

Acta Physiologica Scandinavica

Published monthly for the
Scandinavian Physiological Society,

Vol 110 No 1 September 1980

Editorial Board

P. Kruhoffer
København

M. R. Bergström
Helsinki

J. Jansen
Oslo

Y. Zotterman
Stockholm

U. S. von Euler
(Editor) Stockholm

Editorial office

Acta Physiologica Scandinavica
Karolinska Institutet, Box 80400
S 104 01 Stockholm

The "Acta physiologica scandinavica" are published for the Scandinavian Society for Physiology and contain contributions to Physiology, Medical Chemistry or Pharmacology by Scandinavian authors or from Scandinavian laboratories. The articles are published in English, French or German. Each number consists of about 9 printed sheets, 4 numbers forming a volume. Not more than 3 volumes will appear each year. Subscriptions should be mailed to Acta Physiologica Scandinavica, Karolinska Institutet, S 104 01 Stockholm, Sweden.
Price per volume 170 Sw. Cr.

Manuscripts from Denmark should be sent to Professor P. Kruhoffer
Med. Fysiologiske Inst. B Universitetet, Jullands Marievej 30, DK 2100 København O

Manuscripts from Finland should be sent to Professor M. R. Bergström,
Fysiologiska Institutionen, Siltavuorenpenkki 20, SF-00170 Helsinki 17

Manuscripts from Norway should be sent to Dr. med. Jan Jansen,
Fysiologisk Institutt, Universitetet, Karl Johans Gate 47 N Oslo and

Manuscripts from Sweden should be sent to Professor Y. Zotterman
Wenner Gren Center Konferenssekretariatet, 23 tr Sveavägen 188, S 133 48 Stockholm.

Contents

- 1 JOHANSSON, B. B. & HENNING, M. 6-Hydroxy dopamine and the blood-brain barrier in adult cat: slow rate
 - 8 JÄRHULT J. ANDERSSON, P.-O., HOLST J., MOGHIMZADEH E. & MOBIN, A. On the sympathetic innervation to the cat's liver and its role for hepatic glucose release
 - 13 DAHLSTRÖM, A., BÖÖJ S., HERWALL, P.-O. & LARSSON, P.-A. The effect of chronic nicotine and withdrawal on intra-neuronal dynamics of acetylcholine and related enzymes in a preganglionic neuron system of the rat
 - 21 EKSTRÖM, J. & ELMÉR, M. Compensatory increase of responses to nerve stimulation of the partially denervated rat urinary bladder
 - 31 UDDMAN, R., FAHRENKRUG, J. MALM, L., ALUMETS, J., HÄKANSON, R. & SUNDLER, F. Neuronal VIP in salivary glands: Distribution and release
 - 39 KEMÄNEN, M., KUIKKA, J. HESELIUS, S.-J. SUOLINHA, E.-M., SOUM, O. LÄNGSTRÖM, B. & KÄNTÖ V. Transcapillary exchange and distribution of carbon-11 labelled ethanol in the isolated perfused rat liver
 - 47 KEVALAINEN, T. O. HAKUMÄKI, M. O. K., HYÖDYNMÄÄ, S. J., NÄRHI, M. V. O. & SARAJÄR, H. S. S. Occlusion of pulmonary vein—left atrial junction: Heart rate responses in conscious and anesthetized dogs
 - 53 LARSSON, M., JOHNSON, L., NYLANDER, G. & ÖHMAM, U. Plasma water and ¹⁴Cr-EDTA equilibrium volumes of different tissues in the rat
 - 59 HELLSTRAND, P. & ARNER, A. Contraction of the rat portal vein in hypertonic and isotonic medium: mechanical properties and effects of Mg⁺⁺
 - 69 ARNER, A. & HELLSTRAND P. Contraction of the rat portal vein in hypertonic and isotonic medium: rates of metabolism
 - 77 RICKSTEN, S. E. & THOREN, P. Reflex inhibition of sympathetic activity during volume load in awake normotensive and spontaneously hypertensive rats
 - 83 METSÄ KETELÄ, T. KUOSA, R. & VAPAATALO H. Temporal dissociation between the negative inotropism and the increase in cyclic GMP level induced by choline esters in spontaneously beating rat atria preparations
 - 93 HELLSTRAND, P. & JÄRHULT J. Effects of nine different gastrointestinal polypeptides on vascular smooth muscle in vitro
 - 96 HALLBÄCK, D.-A., JODAL, M. & LUNDGREN, O. Viscous tissue osmolality water and electrolyte transport in the cat small intestine at varying luminal osmolalities
- Short Communications*
- 101 SJÖBERG, H. E., ARVER, S. & BUCHT, E. High concentration of immunoreactive calcitonin of prostatic origin in human semen
 - 103 GRÖLLNER, S. & WALLÉN, P. Does the central pattern generator for locomotion in lamprey depend on glycine inhibition?
 - 107 LUNDBERG, J. M., HÖKFELT T. ÅNGGÅRD, A., KIMMEL, J. GOLDSTEIN, M. & MARKEY K. Coexistence of an avian pancreatic polypeptide (APP) immunoreactive substance and catecholamines in some peripheral and central neurons
 - 111 PELLGRINO, D. & STESJÖ, B. K. The influence of circulating adrenaline on extracellular fluid potassium concentration in the brain
- Appended Supplements*
- Supplementum 483, EDIN, R. The vagal control of the pyloric motor function. A physiological and immunohistochemical study in cat and man
- Supplementum 484, KVIST U. Sperm nuclear chromatin decondensation ability. An in vitro study on ejaculated human spermatozoa

Instructions to authors

Manuscripts (2 copies) should be sent to the National Editor in double spacing on one side paper of size 21 x 30 cm (A 4) with 4 cm margin. A short title (max. 40 letters) may be suggested. An abstract, not exceeding 200 words should be submitted.

In general, a succinct style and restriction to the necessary of documentation and discussion effectively aids in reducing publication time.

References should be given with full title and name of journals, abbreviated in accordance with 4th Ed. of *World List of Scientific Periodicals* with volume number and first and last page numbers.

Figures should not be larger than manuscript pages and sent in as glossy prints in a size larger than that required to reproduction. Lettering should be large enough to permit suitable reduction and preferably of uniform size. Photomicrographs should be calibrated on the print (not enlargement factor in figure text). Figure texts should be assembled on separate sheets.

Tables should be kept at minimum, both in number and size, with text above the table (not on separate sheets). Single numbers in a series should be replaced by mean and S.D. or mean and S.E., in the latter case with number of observations.

Key words (6-10) are recommended in order to facilitate indexing.

For abbreviations, units, and symbols see special list in the Journal and recent articles.

More detailed Instructions to authors are found in NORDIC BIOMEDICAL MANUSCRIPT Instructions & guidelines, published by the Nordic Publication Committee for Medicine, E. G. Svartz Malmberg & R. Goldmann, Universitetsforlaget, Oslo 1978.

The international system of units (SI)

The following symbols and units, recommended by the SI are being used in *Acta Physiologica Scandinavica*. Certain units, not included in SI, will still be permitted.

SI units with recommended symbols

Units	Symbols
kilogramme	kg
second, millisecond	s ms
mole, millimole, micro mole, nanomole, picomole	mol mmol μ mol nmol pmol
meter millimeter micrometer nanometer	m mm μ m nm
candela	cd
steradian	sr
hertz (frequency)	Hz (s^{-1})
newton (force)	N ($kg \cdot m/s^2$)
Pascal (pressure)	Pa (N/m^2)
joule (energy)	J ($N \cdot m$)
watt (effect)	W (J/s)
lumen (lightflow)	lm ($cd \cdot sr$)
lux (illumination)	lx (lm/m^2)

Permitted non SI units

Units	Symbols
gramme	g
minute	min
hour	h
molarity (mol/liter) (calorie) (kilopond) (millimeters of mer- cury) (millibar)	M cal (4 184 J) kp (9.81 N)* mmHg (1.333 mbar) mbar (100 Pa)
curie	Cl
liter milliliter micro- liter	l ml μ l
degree Celsius	C

Conversion factors to be given in Method

Contents

- 1 ALUND, M. Juxtacapsular cell activity during hemorrhage and ischemia as revealed by quinacrine histofluorescence
 - 3 LERNER, U. The effect of dibutyl cyclic AMP and PGE₂ on lysosomal enzyme release and lactate production in relation to bone resorption in vitro
 - 11 MARNIEMI, J., PELTONEN, P., VUORI, L. & HETANEN, E. Lipoprotein lipase of human postheparin plasma and adipose tissue in relation to physical training
 - 17 DELBRO, D. & LISANDER, B. Non-ganglionic cholinergic excitatory pathways in the sympathetic supply to the feline stomach. An afferent system or efference with excitatory axon collaterals?
 - 25 BRISMAR, T. & JEFFERYS, J. G. R. Convulsants (benzylperoxide and pentylenetetrazol) on potential clamped myelinated axons from the rat
 - 33 ABDUL RAHMAN, A. & SJELÖ, B. K. Local cerebral glucose consumption during insulin induced hypoglycemia, and in the recovery period following glucose administration
 - 41 EKELUND, K. & JOHANSSON, C. Output of bile and pancreatic enzymes after test meals with different fat content. Influence of body weight on pancreatic enzyme composition
 - 47 HAUGE, A., THORESEN, M. & WALLØE, L. Changes in cerebral blood flow during hyperventilation and CO₂-breathing measured transcranially in humans by bidirectional, polarized, ultrasonod doppler blood velocitometer
 - 55 HESSA, R., GEORGE, J. C., STEVENS, E. D. & SAARELA, S. Thermoregulatory responses to tyramine in the pigeon
 - 61 SELJÖ, L. C., LIBELJÖ, R., LUNDQVIST, L., TÄGERUD, S. & THIELFF, S. Membrane and biochemical alterations after denervation and during reinnervation of mouse skeletal muscle
 - 187 HELLSTRAND, E. Morphological and histochemical properties of tongue muscles in cat
- Short Communications
- 199 LUNDBERG, J. M., ÅNGGÅRD, A., HÖKFELT, T. & KIMMEL, J. Atrial pancreatic polypeptide (APP) inhibits atropine resistant vasodilation in cat submandibular salivary gland and nasal mucosa: possible interaction with VIP
 - 203 AUER, L. M. & JOHANSSON, B. B. Placental venous constriction during cervical sympathetic stimulation in the cat
 - 207 EDIN, R., LUNDBERG, J. M., LIDBERG, J. & DAHLSTRÖM, A. & AHLMAN, H. Atropine from the contractile motor effects of substance P in the feline pylorus and stomach in vivo
 - 211 SOLLEVI, A. The sensitivity of perfused fat tissue to the antilipolytic action of adenosine
 - 215 NILSSON, H. & FOLKOW, B. Structurally reduced compliance of the venous capacitance vessels by spontaneously hypertensive rats (SHR)
 - 219 HANBERG, M., HEDQVIST, P. & RÅDEGRAN, K. Identification of 15-hydroxy-6,8,11,12-icosatetraenoic acid (15-HETE) as a major metabolite of arachidonic acid in human lung
 - 223 CARLSON, H. & SKOGLUND, C. R. Extracereptive influence from trunk region on monozygotic blood limb reflexes in the cat
- Appendix Supplement
- Supplementum 487 JÄNSSON, E. Diet and muscle metabolism in men with reference to fat and carbohydrate utilization and its regulation

Instructions to authors

Manuscripts (2 copies) should be sent to the National Editor in double spacing on one side paper of size 21 x 30 cm (A 4) with 4 cm margin. A short title (max. 40 letters) may be suggested. An abstract, not exceeding 200 words should be submitted.

In general, a succinct style and restriction to the necessary of documentation and discuss effectively aids in reducing publication time.

References should be given with full title and name of journals, abbreviated in accordance with 4th Ed. of *World List of Scientific Periodicals*, with volume number and first and last page numbers.

Figures should not be larger than manuscript pages and sent in as glossy prints in a size less than that required to reproduction. Lettering should be large enough to permit suitable reduction and preferably of uniform size. Photomicrographs should be calibrated on the print (not enlargement factor in figure text). Figure texts should be assembled on separate sheets.

Tables should be kept at minimum, both in number and size, with text above the table (not separate sheets). Single numbers in a series should be replaced by mean and S.D. or mean \pm S.E., in the latter case with number of observations.

Key words (5-10) are recommended in order to facilitate indexing.

For abbreviations, units, and symbols see special list in the Journal and recent articles.

More detailed instructions to authors are found in *NORDIC BIOMEDICAL MANUSCRIPT INSTRUCTIONS & GUIDELINES*, published by the Nordic Publication Committee for Medicine, E. G. Svartz, Malmberg & R. Goldmann, Universitetsforlaget, Oslo 1978.

The international system of units (SI)

The following symbols and units, recommended by the SI, are being used in *Acta Physiologica Scandinavica*. Certain units, not included in SI, will still be permitted.

SI units with recommended symbols

Units	Symbols
kilogramme	kg
second, millisecond	s ms
mole, millimole, micro mole, nanomole, picomole	mol mmol μ mol nmol pmol
meter millimeter micrometer nanometer	m mm μ m nm
candela	cd
steradian	sr
hertz (frequency)	Hz (s^{-1})
newton (force)	N ($kg\ m/s^2$)
Pascal (pressure)	Pa (N/m^2)
joule (energy)	J ($N\ m$)
watt (effect)	W (J/s)
lumen (lightflow)	lm ($cd\ sr$)
lux (illumination)	lx (lm/m^2)

Permitted non SI units

Units	Symbols
gramme	g
minute	min
hour	h
molarity (mol/liter)	M
(calorie)	cal (4 184 J)
(kilopond)	kp (9.81 N)
(millimeters of mer cury)	mmHg (1,333 mbar)
(millibar)	mbar (100 Pa)
curie	Cl
liter milliliter micro liter	l ml μ l
degree Celsius	C

Conversion factors to be given in Methods

Contents

- STEMKOWICZ, E. & GJEDDE, A. Post-ischemic coma is not effect of different pre-ischemic blood glucose levels on cerebral metabolic recovery after ischemia
- HAMMARSTRÖM, M. Endometrial cholinergic secretory responses during estrous cycle, pregnancy and after estrogen and/or progesterone treatment of the guinea-pig
- JÖHANSSON, M. & WOHLFART, B. Cellular calcium as a determinant of action potential duration in rabbit myocardium
- CLAUSEN, G., HOPE, A., KIRKEBØ, A., TYBSE BØTH, I. & AUKLAND, K. Distribution of blood flow in the dog kidney II. Saturation rates of liver diffusible tracers versus uptake of $15 \mu\text{m}$ microsphere during vasodilation and vasoconstriction
- FELLENZ, E., HEDBERG, R. & KARLSSON, M. The effect of β -receptor blockade on adrenaline-induced changes in redox and energy state in its vesicle lumen and in axolemma of the rat in vivo
- LIPS, P. E., PETERSSON, B. & EFENDIĆ, S. Effects of short-term and prolonged infusions of somatostatin on endocrine pancreas, body weight and food intake in rats
- HOHTOLA, E., SAARELA, S. & HISSA, R. Effects of blood pressure manipulations on shivering thermogenesis in the pigeon
- HAUKIO, S. & AMTORP, O. Regional blood flow during reactive hyperemia in canine myocardium as determined by local washout of Xe-133
- QJÖRSTRUP, P. Taste and chewing as stimuli for the secretion of amylase from the parotid gland of the rabbit
- KOVUVALO, F. Degradation of TRF immunoreactivity in rat hypothalamus, extrahypothalamic brain and pancreas
- HULTHÉN, U. L., DYMLING, J.-F. & HÖGFELT, B. Kinase in relation to kallikrein activity kininogen, electrolytes, aldosterone and catecholamines in urine from normal individuals
- 315 AARS, H. Central interaction between reflex responses to activity in aortic nerve A- and C-fibres

Short Communications

- 321 FUXE, K., GANTEN, D., KÖHLER, C., SCHÖLL, B. & SPECK, G. Evidence for differential localization of angiotensin-I converting enzyme and renin in the corpus striatum of rat
- 325 ROSELL, S. Experimental evidence for neurotensin or a metabolite being a hormone
- 326 RÖKAEUS, Å. & McDONALD, T. Release of neurotensin-like immunoreactivity (NTLI) from rat intestine following administration of bombesin
- 327 THOR, K., RÖKAEUS, Å., KAGER, L. & ROSELL, S. (Gln)-Neurotensin and gastrointestinal motility in man
- 329 KJHL, B., RÖKAEUS, Å., ROSELL, S. & OLBE, L. Inhibition of pentagastrin stimulated gastric acid secretion and rise in the plasma concentration of neurotensin-like immunoreactivity (NTLI) by intraduodenal oleic acid in man
- 330 WIKLUND, B., RÖKAEUS, Å., HALLBERG, D. & ROSELL, S. Plasma NTLI after administration of fat to obese volunteers and patients operated with jejuno-ileal bypass
- 331 HEDQVIST, P., DAHLÉN, S. E., GUSTAFSSON, L., HAMMARSTRÖM, S. & SAMUELSSON, B. Biological profile of leukotrienes C_4 and D
- 333 CHAPMAN, A., INGVAR, M. & STESJÖ, B. K. Free fatty acids in the brain in bicuculline-induced status epilepticus

Appendix Supplement

- Supplementum 488. RIDDERSTRÅLE, Y. Intracellular localization of carbonic anhydrase in some vertebrate nephrons.

Instructions to authors

Manuscripts (2 copies) should be sent to the National Editor in double spacing on one side paper of size 21 x 30 cm (A 4) with 4 cm margin. A short title (max. 40 letters) may be suggested. An abstract, not exceeding 200 words should be submitted.

In general, a succinct style and restriction to the necessary of documentation and discuss effectively aids in reducing publication time.

References should be given with full title and name of journals, abbreviated in accordance with 4th Ed. of *World List of Scientific Periodicals* with volume number and first and last page numbers.

Figures should not be larger than manuscript pages and sent in as glossy prints in a size larger than that required to reproduction. Lettering should be large enough to permit suitable reduction and preferably of uniform size. Photomicrographs should be calibrated on the print (not enlargement factor in figure text). Figure texts should be assembled on separate sheets.

Tables should be kept at minimum, both in number and size, with text above the table (not separate sheets). Single numbers in a series should be replaced by mean and S.D. or mean \pm S.E., in the latter case with number of observations.

Key words (5-10) are recommended in order to facilitate indexing.

For abbreviations, units, and symbols see special list in the Journal and recent articles.

More detailed instructions to authors are found in NORDIC BIOMEDICAL MANUSCRIPT Instructions & guidelines, published by the Nordic Publication Committee for Medicine, E. G. Svartz, Malmberg & R. Goldmann, Universitetsforlaget, Oslo 1978.

The International system of units (SI)

The following symbols and units, recommended by the SI, are being used in *Acta Physiologica Scandinavica*. Certain units, not included in SI, will still be permitted.

SI units with recommended symbols

Units	Symbols
kilogramme	kg
second, millisecond	s ms
mole, millimole, micromole, nanomole, picomole	mol mmol μ mol nmol pmol
meter millimeter micrometer nanometer	m mm μ m nm
candela	cd
steradian	sr
hertz (frequency)	Hz (s^{-1})
newton (force)	N ($kg\ m/s^2$)
Pascal (pressure)	Pa (N/m^2)
joule (energy)	J ($N\ m$)
watt (effect)	W (J/s)
lumen (lightflow)	lm ($cd\ sr$)
lux (illumination)	lx (lm/m^2)

Permitted non SI units

Units	Symbols
gramme	g
minute	min
hour	h
molarity (mol/liter) (calorie) (kilopond) (millimeters of mercury) (millibar)	M cal (4.184 J) kp (9.81 N) mmHg (1.333 mbar) mbar (100 Pa)
curie	Cl
liter milliliter micro liter	l ml μ l
degree Celsius	C

Conversion factors to be given in Method

- AMTORP O. Estimation of capillary permeability of inulin, sucrose and mannitol in rat brain cortex
- FASTH, S. & HULTÉN, L. & NORDGREN, S. Adjustments of hepatic and small intestine blood flow on selective vasoconstrictor fibre stimulation
- BECH, C. & JOHANSEN, K. Blood flow changes in the duck during thermal panting
- UYELIUS, B. & GABELLA, G. Relation between cell length and force production in urinary bladder smooth muscle
- LARSEN, J. A. & THOMSEN, O. O. Vanadate-induced oligemia and vasoconstriction in the cat
- HANDEBO, J. E., EDVINGSSON, L. & JOHANSSON, B. B. Cardiovascular sympathetic denervation and blood-brain barrier function in conscious rats
- SJÖQVIST A. & LJUNG, B. Dissociation of electrical and mechanical activity caused by vibrations in the spontaneously active smooth muscle of the rat portal vein
- LEHTO, H. & TIRRI, M. Characterization of contractions in mechanically disaggregated myocardial cells from the rat
- UYNÄS-WALLÉN, K., EFENDIC, S., JOHANSSON, C., SJÖQVIST, L. & CRANWELL, P. D. Effect of leucokinin-pH on the release of acetylcholine and gastrin into arterial, biliary and fecal pouches of conscious dogs
- GUSTAFSSON, L., HEDQVIST P. & LUNDGREN, G. Pre- and postfunctional effects of prostaglandin E prostaglandin synthetase inhibitors and atropine on cholinergic neurotransmission in guinea pig ileum and bovine iris
- RICKSTEN, S. E., YAO, T., LJUNG, B. & THORÉN, P. Distensibility of left striated in normotensive and spontaneously hypertensive rats
- FLEMLSTRÖM, G. & GARNER, A. Stimulation of gastric acid and bicarbonate secretions by calcium in guinea pig stomach and amphibian isolated mucosa
- Short Communications*
- WAHLSTRÖM, A. & TERENIUS, L. Factor in human CSF with apparent morphine-antagonistic properties
- THOREN, P. Characteristics of right ventricular receptors with non-modulated vagal efferents in the cat
- REHNCRONA, S., ROSEN, I. & SIESJÖ, B. K. Excessive cellular acidosis: an important mechanism of neuronal damage in the brain?
- BRISMAR, T. Potential clamp of demyelinated rat nerve fibres
- HEDQVIST P. Autoinhibition versus transsynaptic control of norepinephrine secretion in the rabbit kidney
- TÖREBJÖRK, H. E. & OCHOA, J. L. Specific sensations evoked by activity in single identified sensory units in man
- MALMSTEN C. L., PALMBLAD J., UDÉN A. M., RÄDMARK, O., ENGSTEDT L. & SAMUELSSON, B. Leukotriene B₄ A highly potent and stereospecific factor stimulating migration of polymorphonuclear leucocytes

Appendix Supplement

- Supplementum 429 GUSTAFSSON, L. E. Studies on modulation of transmitter release and effector responsiveness in autonomic cholinergic neurotransmission

Instructions to authors

Manuscripts (2 copies) should be sent to the National Editor in double spacing on one paper of size 21 x 30 cm (A 4) with 4 cm margin. A short title (max. 40 letters) may be included. An abstract, not exceeding 200 words should be submitted.

In general, a succinct style and restriction to the necessary of documentation and of references aids in reducing publication time.

References should be given with full title and name of journals, abbreviated in accordance with 4th Ed. of *World List of Scientific Periodicals*, with volume number and first and last page numbers.

Figures should not be larger than manuscript pages and sent in as glossy prints in a size larger than that required to reproduction. Lettering should be large enough to permit suitable reduction and preferably of uniform size. Photomicrographs should be calibrated on the print enlargement factor in figure text. Figure texts should be assembled on separate sheets.

Tables should be kept at minimum, both in number and size, with text above the table (on separate sheets). Single numbers in a series should be replaced by mean and S.D. or mean \pm S.E., in the latter case with number of observations.

Key words (5-10) are recommended in order to facilitate indexing.

For abbreviations, units, and symbols see special list in the Journal and recent articles.

More detailed Instructions to authors are found in NORDIC BIOMEDICAL MANUSCRIPT INSTRUCTIONS & GUIDELINES, published by the Nordic Publication Committee for Medicine, E. G. Svartz, Malmberg & R. Goldmann, Universitetsforlaget, Oslo 1978.

The international system of units (SI)

The following symbols and units, recommended by the SI, are being used in *Acta Physiologica Scandinavica*. Certain units, not included in SI, will still be permitted.

SI units with recommended symbols

Units	Symbols
kilogramme	kg
second, millisecond	s ms
mole, millimole, micromole, nanomole, picomole	mol mmol μ mol nmol pmol
meter millimeter micrometer nanometer	m mm μ m nm
candela	cd
steradian	sr
hertz (frequency)	Hz (s^{-1})
newton (force)	N ($kg \ m/s^2$)
Pascal (pressure)	Pa (N/m^2)
joule (energy)	J ($N \ m$)
watt (effect)	W (J/s)
lumen (lightflow)	lm ($cd \ sr$)
lux (illumination)	lx (lm/m^2)

Permitted non SI units

Units	Symbols
gramme	g
minute	min
hour	h
molarity (mol/liter) (calorie) (kilopond) (millimeters of mercury) (curie) (millibar)	M cal (4.184 J) kp (9.81 N) mmHg (1.333 mbar) mbar (10 ⁵ Pa)
curie	Cl
liter milliliter microliter	l ml μ l
degree Celsius	C

Conversion factors to be given in Method

6-Hydroxydopamine and the blood-brain barrier in adult conscious rats

BARBRO ■ JOHANSSON and MATTS HENNING

Departments of Neurology and Pharmacology University of Göteborg, Sweden

JOHANSSON B ■ & HENNING, M., 6-hydroxydopamine and the blood-brain barrier in adult conscious rats. *Acta Physiol Scand* 1980, 110: 1-4. Received 3 Sept. 1979. ISSN 0001-6772. Departments of Neurology and Pharmacology University of Göteborg, ■ edes.

6-hydroxydopamine (6-OHDA), 15 or 50 mg kg⁻¹ given as bolus injection to adult conscious rats with aortic catheter rapidly increased mean arterial pressure by 70-78 mmHg. The pressure returned to normal within 40-60 min. The cerebrovascular permeability in rats given 6-OHDA and sacrificed 10 or 60 min later was enhanced as indicated by extravasation of Evans blue albumin and significant increase of [¹²⁵I] human serum albumin content in brain tissue compared to control rats. When the increase in blood pressure was diminished by phentolamine, 6-OHDA treated rats did not differ from controls. It is concluded that the blood pressure elevation induced by i.v. 6-OHDA facilitates the entry of the drug into the brain parenchyma.

Key words: Albumin, blood-brain barrier, hypertension, 6-OHDA.

6-hydroxydopamine (6-OHDA) is extensively used in experimental physiology and pharmacology because of its selective cytotoxic action on catecholamine-containing neurons. It is generally considered that systemically administered 6-OHDA does not pass the blood-brain barrier (BBB) in adult animals (Laverly, Stairman & Vogt 1965; Thoenen 1977; Kostrowa & Jacobowitz 1974). However it has been reported that the BBB does not completely protect central noradrenergic terminals from the neurotoxic action of large doses (100 mg kg⁻¹) of 6-OHDA given to adult rats (Sachs 1973; Sachs & Johnson 1973). An important consideration in this connection is the fact that an injection of 6-OHDA elicits powerful sympathomimetic effects including a rapid rise of the arterial blood pressure (de Champlain & Nadeau 1971; de Champlain & Anagnostou 1977). It is well known that an abrupt increase in blood pressure can lead to a transient dysfunction of the BBB allowing passage of substances including macromolecules into the brain both in conscious and anesthetized animal (Johansson et al. 1970; Johansson & Hemming 1976; Johansson 1978). Based on these considerations we have therefore examined the BBB function in mac-

romolecules after i.v. injections of 6-OHDA in adult conscious rats with the aim of elucidating whether the blood pressure increase induced by i.v. 6-OHDA can lead to a BBB dysfunction which may allow the drug to pass into the brain also in adult animals.

MATERIAL AND METHODS

Indwelling catheters were inserted under isoflurane anesthesia (Brietal Sodium 50 mg kg⁻¹) into the abdominal aorta via the left femoral artery and in the right jugular vein in 40 male Sprague Dawley rats weighing 200-250 g. The free end of the catheter was exteriorized on the back of the neck. Two days later the aortic catheter was connected to a transducer and mean arterial pressure (MAP) recorded in awake, unrestrained rats. Repeated determinations of arterial PCO₂, PO₂ and pH were made. [¹²⁵I] human serum albumin (HSA, 100 µC kg⁻¹) and Evans blue (2 ml kg⁻¹ of 2% solution in saline) were given as indicators of the BBB function. Evans blue binds to serum albumin *in vivo* and is easily detected macroscopically providing convenient gross evaluation of leakage. In addition the staining of the blood provides an indication of the effectiveness of the perfusion (see below). Thereafter 6-OHDA (15 or 50 mg kg⁻¹ freshly dissolved in 0.9% sodium chloride solution containing ascorbic acid 0.2 mg ml⁻¹) was given as bolus injec-

Instructions to authors

Manuscripts (2 copies) should be sent to the National Editor in double spacing on one side paper of size 21 x 30 cm (A 4) with 4 cm margin. A short title (max. 40 letters) may be included. An abstract, not exceeding 200 words should be submitted.

In general, a succinct style and restriction to the necessary of documentation and discussion effectively aids in reducing publication time.

References should be given with full title and name of journals, abbreviated in accordance with 4th Ed. of *World List of Scientific Periodicals*, with volume number and first and last numbers.

Figures should not be larger than manuscript pages and sent in as glossy prints in a size than that required to reproduction. Lettering should be large enough to permit suitable reduction and preferably of uniform size. Photomicrographs should be calibrated on the print (enlargement factor in figure text). Figure texts should be assembled on separate sheets.

Tables should be kept at minimum, both in number and size, with text above the table (separate sheets). Single numbers in a series should be replaced by mean and S.D. or S.E., in the latter case with number of observations.

Key words (5-10) are recommended in order to facilitate indexing.

For abbreviations, units, and symbols see special list in the Journal and recent articles.

More detailed instructions to authors are found in *NORDIC BIOMEDICAL MANUSCRIPT INSTRUCTIONS & GUIDELINES*, published by the Nordic Publication Committee for Medicine, P. G. Svartz, Melnberg & R. Goldmann, Universitetsforlaget, Oslo 1978.

The international system of units (SI)

The following symbols and units, recommended by the SI, are being used in *Acta Physiologica Scandinavica*. Certain units, not included in SI, will still be permitted.

SI units with recommended symbols

Units	Symbols
kilogramme	kg
second, millisecond	s ms
mole, millimole, micromole, nanomole, picomole	mol mmol μ mol nmol pmol
meter millimeter micrometer nanometer	m mm μ m nm
candela	cd
steradian	sr
hertz (frequency)	Hz (s^{-1})
newton (force)	N ($kg \cdot m/s^2$)
Pascal (pressure)	Pa (N/m^2)
joule (energy)	J ($N \cdot m$)
watt (effect)	W (J/s)
lumen (lightflow)	lm ($cd \cdot sr$)
lux (illumination)	lx (lm/m^2)

Permitted non SI units

Units	Symbols
gramme	g
minute	min
hour	h
molarity (mol/liter)	M
(calorie)	cal (4.184 J)
(kilopond)	kp (9.81 N) ^a
(millimeters of mercury)	mmHg
(curie)	(1.333 mbar)
(millibar)	mbar (100 Pa)
curie	Cl
liter milliliter microliter	l ml μ l
degree Celsius	C

Conversion factors to be given in Methods

3-Hydroxydopamine and the blood brain barrier in adult conscious rats

JARBRÖ B. JOHANSSON and MATTS HENNING

Departments of Neurology and Pharmacology University of Göteborg, Sweden

JOHANSSON B. & HENNING, M. 6-hydroxydopamine and the blood-brain barrier in adult conscious rats. *Acta Physiol Scand* 1980, 110: 1-4. Received 3 Sept. 1979. ISSN 0001-6772. Departments of Neurology and Pharmacology University of Göteborg, Sweden.

6-hydroxydopamine (6-OHDA), 15 or 30 mg/kg given as bolus i.v. injections to adult conscious rats with aortic catheter rapidly increased mean arterial pressure by 70-78 mmHg. The pressure returned to normal within 40-60 min. The cerebrovascular permeability in rats given 6-OHDA and sacrificed 10 or 60 min later was enhanced as indicated by extravasation of Evans blue albumin and significant increase of ¹²⁵I human serum albumin content in brain tissue compared to control rats. When the increase in blood pressure was diminished by phentolamine 6-OHDA treated rats did not differ from control. It is concluded that the blood pressure elevation induced by i.v. 6-OHDA facilitates the entry of the drug into the brain parenchyma.

Key words: Albumin, blood-brain barrier, hypertension, 6-OHDA.

6-hydroxydopamine (6-OHDA) is extensively used in experimental physiology and pharmacology because of its selective cytotoxic action on catecholamine-containing neurons. It is generally considered that systemically administered 6-OHDA does not pass the blood-brain barrier (BBB) in adult animals (Laerty, Sharman & Vogt 1965; Thoenen 1977; Kostrewa & Jacobowitz 1974). However it has been reported that the BBB does not completely protect central noradrenergic terminals from the neurotoxic action of large doses (100 mg/kg) of 6-OHDA given to adult rat (Sachs 1973; Sachs & Johansson 1973). An important consideration in this connection is the fact that an injection of 6-OHDA elicits powerful sympathomimetic effects including rapid rise of the arterial blood pressure (de Champlain & Nadeau 1971; de Champlain & Anagnostou 1977). It is well known that an abrupt increase in blood pressure can lead to a transient dysfunction of the BBB allowing passage of substances including macromolecules into the brain both in conscious and anesthetized animals (Johansson et al 1970; Johansson & Hemming 1976; Johansson 1978). Based on these considerations we have therefore examined the BBB function to mac-

romolecules after i.v. injections of 6-OHDA in adult, conscious rats with the aim of elucidating whether the blood pressure increase induced by i.v. 6-OHDA can lead to a BBB dysfunction which may allow the drug to pass into the brain also in adult animals.

MATERIAL AND METHODS

Indwelling catheters were inserted under methohexital anaesthesia (Brevital Sodium, 30 mg/kg) into the abdominal aorta via the left femoral artery and in the right jugular vein in 40 male Sprague-Dawley rats weighing 200-350 g. The free end of the catheter was exteriorized on the back of the neck. Two days later the aortic catheter was connected to a transducer and mean arterial pressure (MAP) recorded in awake unrestrained rats. Repeated determinations of arterial PCO₂, PO₂ and pH were made using human serum albumin (HSA, 100 µCi/kg) and Evans blue (2 ml/kg of 2% solution in saline) were given as indicators of the BBB function. Evans blue binds to serum albumin *in vivo* and is easily detected macroscopically providing convenient gross evaluation of leakage. In addition the staining of the blood provides an indication of the effectiveness of the perfusion (see below). Thereafter 6-OHDA (15 or 30 mg/kg freshly dissolved in 0.9% sodium chloride solution containing ascorbic acid 0.2 mg/ml) was given i.v. as bolus injec-

Table 1 Mean arterial pressure (MAP) before and after the injection of 6-hydroxydopamine (6-OHDA) solvent (controls) to conscious unrestrained rats

PaCO₂, PaO₂ and pH before the injection of the drug. Mean values \pm S.E.

Experimental group	n	Initial MAP	Maximum MAP	MAP 10 min after injection	MAP 60 min after injection	PaCO ₂ (kPa)	PaO ₂ (kPa)	pH
<i>10 min</i>								
Control	6	105 \pm 3	—	103 \pm 4	—	4.6 \pm 0.1	11.4 \pm 0.1	7.34 \pm 0.1
6-OHDA 15 mg/kg	6	98 \pm 1	171 \pm 1	123 \pm 5	—	4.3 \pm 0.1	11.7 \pm 0	7.33 \pm 0.1
6-OHDA 50 mg/kg	6	109 \pm 4	187 \pm 5	154 \pm 4	—	4.4 \pm 0	11.8 \pm 0.1	7.35 \pm 0.1
<i>60 min</i>								
Control	8	105 \pm 6	—	115 \pm 6	117 \pm 7	4.3 \pm 0.1	11.8 \pm 0.1	7.35 \pm 0.1
6-OHDA 50 mg/kg	6	106 \pm	176 \pm 5	160 \pm 4	101 \pm 5	4.5 \pm 0.1	11.5 \pm 0.1	7.36 \pm 0.1
Phentolamine 5 mg/kg	8	96 \pm 3	144 \pm 4	104 \pm 4	109 \pm 6	4.4 \pm 0.1	11.6 \pm 0.1	7.36 \pm 0.1
6-OHDA 50 mg/kg								

tion. Control rats received Evans blue ¹²⁵I-HSA and the solvent but no 6-OHDA. One group of rats was pretreated with 5 mg/kg of the alpha-adrenoreceptor antagonist phentolamine 10–15 min before the injection of 6-OHDA to minimize the rise in blood pressure. 10 or 60 min after the administration of 6-OHDA the rats were given 30 mg pentobarbital i.v. and the brains were perfused *in situ* with saline through the heart for 1 min to remove the tracers from cerebral blood vessels. Immediately before the start of the perfusion a blood sample was taken for ¹²⁵I-HSA determination. The brains were divided in telencephalon, diencephalon, mesencephalon, cerebellum and pons+medulla oblongata (Zeman & Jones 1963). The samples were weighed and the radioactivity determined in a scintillation counter. The brain activity was calculated as a percentage of the activity in brain and blood, i.e. 100 \times [CPM/mg brain tissue over CPM/mg blood]. Statistical differences were evaluated with Wilcoxon rank sum test.

RESULTS

MAP before and after 6-OHDA as well as PaCO₂, PaO₂ and pH immediately prior to the blood pressure increase are shown in Table 1. The blood pressure elevation induced by 6-OHDA had a rapid onset and long duration. Maximum MAP was reached within 5–25 s and the pressure returned to the initial level within 40–60 min after the high dose (50 mg/kg). No change in blood pressure was observed in animals treated with solvent only. Phentolamine induced an initial drop in blood pressure of 40–50 mmHg but at the time of 6-OHDA injection the pressure had stabilized on a level 15 mmHg lower than initially. The pressure response to 6-OHDA was markedly reduced in the

Table 2 ¹²⁵I-HSA leakage in the brain after i.v. injection of 6-hydroxydopamine (6-OHDA) in conscious unrestrained rats

The relative activity is shown as a ratio of activity between brain and blood, i.e. (CPM/mg brain tissue over CPM/mg blood) \times 100. Mean values \pm S.E.

Experimental group		Telencephalon	Diencephalon	Mesencephalon	Pons Medulla obl.	Cerebellum
<i>10 min</i>						
Control	6	0.0 \pm 0.01	0.0 \pm 0.01	0.0 \pm 0.01	0.0 \pm 0.01	0.0 \pm 0.01
6-OHDA 15 mg/kg	6	0.07 \pm 0.01	0.09 \pm 0.01	0.08 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.01
6-OHDA 50 mg/kg	6	0.13 \pm 0.03	0.17 \pm 0.04	0.13 \pm 0.03	0.11 \pm 0.03	0.1 \pm 0.01
<i>60 min</i>						
Control	8	0.08 \pm 0.01	0.08 \pm 0.01	0.09 \pm 0.01	0.09 \pm 0.01	0.10 \pm 0.01
6-OHDA 50 mg/kg	6	0.14 \pm 0.01	0.19 \pm 0.02	0.13 \pm 0.01	0.14 \pm 0.01	0.10 \pm 0.01
Phentolamine 5 mg/kg	8	0.09 \pm 0.01	0.11 \pm 0.01	0.09 \pm 0.01	0.08 \pm 0.01	0.09 \pm 0.01
6-OHDA 50 mg/kg						

$P < 0.05$, $P < 0.01$ for differences from control rats

group of rats (see Table 1). 14 out of 18 brains from rats given 6-OHDA showed small scattered dots of Evans blue-albumin extravasation. In 7 of these rats—4 belonged to rats given the low dose 6-OHDA—such big areas were few i.e. 5 or less. Evans blue-albumin leakage was not observed in controls or in rats pretreated with phentolamine. Except in the latter group, the brains from rats given 6-OHDA had significantly higher radioactivity levels than controls (see Table 1).

CONCLUSIONS

It is evident from our results that the cerebrovascular permeability to albumin is enhanced after intravenous administration of 6-OHDA in the present doses. The fact that rats pretreated with phentolamine did not differ from controls, indicates that it is the increase in blood pressure rather than any toxic effect of 6-OHDA that is of importance. Moreover the multifocal appearance of the extravasation is the typical pattern seen in acute hypertension induced by other means (cf. Johansson 1976). The passage of 6-OHDA into the brain is not exclusively prevented by the morphological barrier but probably also by the enzymatic barrier to catecholamines in the endothelial cells i.e. monoamine oxidase and aromatic aminoacid decarboxylase (Bertler et al. 1966; Hardebo et al. 1979). In the presence of a transient BBB dysfunction it seems that this enzymatic barrier is not sufficient to prevent catecholamines from entering the brain, as indicated by the marked effect on cerebral metabolism induced by noradrenaline and adrenaline after hypertensive or hyperosmotic BBB opening (MacKenzie et al. 1976; Abdul-Rahman et al. 1979) and the presence of amines in the brain parenchyma (Hardebo et al. 1979). There is evidence that adrenaline can pass into the brain at high level of blood pressure to a greater extent than would be expected from the macroscopical leakage of Evans blue-albumin (Abdul-Rahman et al. 1979). Hence it is likely that 6-OHDA did enter the brain parenchyma in our experiments. It should be emphasized that transitory BBB dysfunction may occur at relatively moderate blood pressure levels. During normotension, MAP levels exceeding 160 mmHg normally lead to an increased permeability and during hypertensive protein leakage occurs at even lower pressures (Johansson & Linder 1978). Therefore consideration of arterial pressure is essential

in pharmacological and physiological studies using drugs with potential toxic or metabolic effects if allowed access to the brain cells during a transient BBB dysfunction. In this connection it should be recalled that transient increases in arterial pressure may result in asymmetrical BBB dysfunction if one carotid artery is ligated previously—the brain tissue on the ligated side being protected whereas signs of BBB dysfunction may develop on the non-ligated side (Johansson & Henning 1976; Johansson, Linder & Borenstein 1978). Thus it is preferable to use a catheter in a femoral artery or in the abdominal aorta in this kind of experiments.

This study was supported by grants from the Swedish Medical Research Council (projects No. 2363 and 4968) and from the Swedish National Association against Heart and Chest Diseases.

REFERENCES

- ABDUL RAHMAN A, DAHLGREN N, JOHANSSON B B & SIESJÖ B K. 1979 Increase in local cerebral blood flow induced by circulating 'adrenaline' involvement of blood-brain barrier dysfunction. *Acta Physiol Scand* 107: 227-232.
- BERTLER, A, FALCK, B, OWMAN C. & ROSEN-GREN E. 1966 Localization of monoaminergic blood-brain barrier mechanisms. *Pharmacol Rev* 18: 369-385.
- de CHAMPLAIN J & van AMERINGEN H R. 1977 Regulation of blood pressure by sympathetic nerve fibres and adrenal medulla in normotensive and hypertensive rats. *Circ Res* 31: 617-628.
- de CHAMPLAIN J & NADEAU R. 1971 6-hydroxydopamine, 6-hydroxydopa and degeneration of adrenergic nerves. *Fed Proc* 30: 877-885.
- HARDEBO J E, EDVINGSSON L, MACKENZIE, E T & OWMAN C. 1979 Histofluorescence study on monoamine entry into the brain before and after opening of the blood-brain barrier by various mechanisms. *Acta Neuropath (Berl)* 47: 145-149.
- JOHANSSON B B. 1976 Brain barrier pathology in acute arterial hypertension. In: *Transport phenomena in the nervous system* (ed O Lavi, L Batistini and A. Lajtha). *Adv Exp Med Biol* 69: 517-527. Plenum Press, New York.
- JOHANSSON B B. 1978 Effect of an acute increase of the intravascular pressure on the blood-brain barrier: A comparison between conscious and anaesthetized rats. *Stroke* 9: 588-590.
- JOHANSSON B B & HENNING M. 1976 The clinical effect of acute blood pressure increase in conscious rats. *Acta Physiol Scand* 98: 376-378.
- JOHANSSON B B, LI C L, OLSSON Y & KLATZKO I. 1970 The effect of acute arterial hypertension on the blood-brain barrier to protein tracers. *Acta Neuropath (Berl)* 16: 117-124.

- JOHANSSON B B & LINDER L E 1978 Cerebrovascular permeability to protein in the rat during nitrous oxide anesthesia at various blood pressure levels. *Acta Anaesth Scand* 22 463-466
- JOHANSSON B B LINDER L E & BORENSTEIN P 1978 Cerebrovascular permeability in acute arterial hypertension. Effect of unilateral ligation of the common carotid artery. *Eur Neurol* 17 205-210
- KOSTRZEWA R M & JACOBOWITZ D M 1974 Pharmacological actions of 6-hydroxydopamine. *Pharmacol Rev* 26 199-288
- LAVERTY R, SHARMAN D F & VOGT M 1965 Action of 4-5-trihydroxyphenylethylamine on the storage and release of noradrenaline. *Br J Pharmacol* 24 549-560
- MACKENZIE E T McCULLOCH J O'KEANE V PICKARD J D & HARPER A M 1976 Cerebral circulation and norepinephrine. Relevance of the blood brain barrier. *Amer J Physiol* 31 483-498
- SACHS C 1973 Development of the blood-brain barrier for 6-hydroxydopamine. *J Neurochem* 20 1753-1760
- SACHS C & JONSSON G 1973 Changes in cerebellar noradrenergic neurons after systemic 6-hydroxydopamine administration. *J Neurochem* 1517-1524
- ZEMAN W & INNES J R M 1963 *Craige neuroanatomy of the rat*. Academic Press New York and London

In the sympathetic innervation to the cat's liver and its role for hepatic glucose release

JÄRHULT P-O ANDERSSON J HOLST
MOGHIMZADEH and A NOBIN

Departments of Physiology Histology and Surgery University of Lund Sweden
ii Department of Medical Physiology C University of Copenhagen Denmark

JÄRHULT J ANDERSSON P-O HOLST J MOGHIMZADEH E. & NOBIN A.
On the sympathetic innervation to the cat liver and its role for hepatic glucose release
Acta Physiol Scand 1980, 110: 5-11 Received 1 Nov 1979 ISSN 0001-6772. Departments
of Physiology Histology and Surgery University of Lund, Sweden and Department
of Medical Physiology C, University of Copenhagen, Denmark.

Morphology and function of the adrenergic innervation of the liver were studied in cats. Fluorescence microscopy revealed dense network of adrenergic nerve fibres in association with interlobular vessels and sparse, but unequivocal innervation of the hepatocytes. These parenchymal adrenergic nerve fibres were more frequent in kittens (2 months old) than in adult cats. Electrical stimulation of the hepatic sympathetic nerves in the adult adrenalectomized cat evoked small but insignificant increment (1-2 mM) of arterial plasma glucose concentration. When both hepatic and pancreatic sympathetic nerves were stimulated simultaneously arterial plasma glucose concentration increased significantly by about 6 mM. We conclude that the pronounced hyperglycemic effect of activation of the sympathetic nervous system in the cat is mediated mainly via an adrenergic influence on the release of insulin and glucagon from the pancreas. The sympathetic innervation of the cat liver parenchyma seems to contribute to the hyperglycemia to minor extent only.

Key words: Sympatho-adrenal system, catecholamines, fluorescence microscopy, glucose release, insulin, glucagon.

The mammalian liver is richly supplied with both sympathetic and parasympathetic nerve fibres which enter the liver mainly via a plexus surrounding the hepatic artery. As far as the sympathetic nerve fibres are concerned, previous morphological studies have reported that these nerves are distributed almost exclusively to the vasculature of the liver (Ugvary & Donath 1969; Kyösola 1978) and a number of physiological studies have in great detail described the effects of sympathetic stimulation on the hepatic resistance, capacitance and exchange vessel (see review by Greenway & Stark 1971). However, during the last decade studies on different laboratory animals have suggested that the sympathetic nerves to the liver actually can influence also glucose homeostasis in so far that an increased activity in these nerves leads to an increment of hepatic glucose release (Shimazu & Amann 1968; Edwards 1971, 1972; Shimazu & Fujimoto 1971; Edwards & Silver 1972; Järhult 1975; Laitt & Wong 1978; Laitt 1979; Seydoux et

al 1979). Recently such an effect of stimulating the hepatic sympathetic nerves has also been shown in man (Nobin et al. 1977; Järhult et al. 1979) and refined histochemical techniques have demonstrated a sympathetic innervation of liver cells in monkey and in man (Falck et al. 1975; Nobin et al. 1978).

This study was undertaken in order to correlate morphology and function of sympathetic nerves in the cat liver. Our results indicate that the sympathetic hepatic nerves play a minor role in the control of hepatic glucose release in cats and that activation of the sympathetic innervation promotes glucose output from the liver mainly via adjustments of glucagon and insulin release from the endocrine pancreas.

METHODS

Stimulation experiments. 11 cats with an average b.wt. of 3.3 kg were used for the stimulation expts. The animals were fed on standard pellet diet but food was withheld

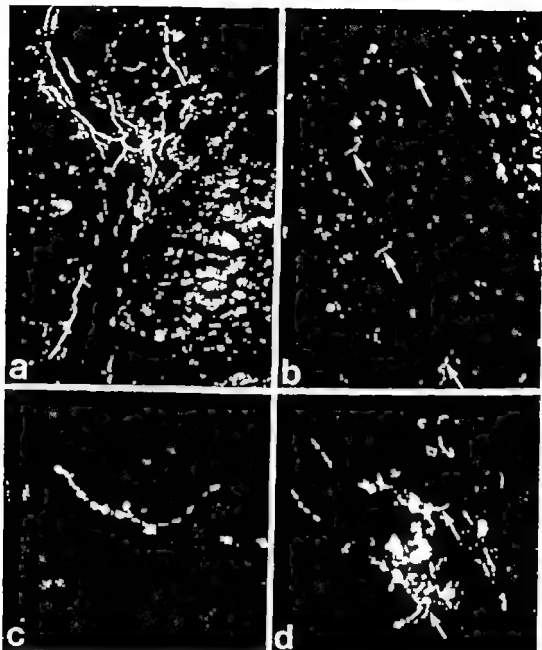


Fig 1 *a-c* are photomicrographs from the liver parenchyma of a two-month-old cat, whereas *d* is taken from the endocrine pancreas of an adult cat. (*a*) represents the typical noradrenergic innervation to a major artery ($\times 100$). (*b*) shows the sparse parenchymal innervation. Noradrenaline-containing nerve fibres are pointed out with arrows ($\times 120$). (*c*) shows a noradrenaline-containing varicosed nerve fibre running in close contact with the hepatocytes ($\times 180$). (*d*) shows noradrenaline-containing nerve fibres in an islet of Langerhans (marked with arrows). Note also the specific fluorescence of the endocrine cells ($\times 180$).

for at least 12 h before the expt. Anesthesia was induced with ether and maintained with chloralose (50 mg/kg) and urethane (100 mg/kg) given i.v. A tracheal cannula was inserted. The abdomen was opened with a midline incision and the adrenal glands were removed bilaterally. The right axillary artery was cannulated and connected to Statham P23AC transducers for recording of mean and pulsatile arterial blood pressure. Heart rate was recorded with a tachograph triggered by the systolic pressure wave. In 10 cats the hepatic sympathetic nerves were carefully dis-

sected free from the hepatic artery close to its entrance at the liver hilum. The nerve fibres were then cut and the distal ends placed in a bipolar platinum ring electrode connected to a Grass stimulator. All nerve fibres along the gastroduodenal artery were cut in order to prevent any stimulation of the sympathetic nerves to the pancreas.

The animal were allowed to recover from surgery during 30–45 min. The hepatic sympathetic nerves were then stimulated for 10 min with 10 Hz, 10 V and 1 ms (6 cats) or for 70 min with 70 Hz, 70 V and 1 ms (4 cats).

Table 1. Tissue concentrations of noradrenaline, adrenaline and dopamine in the cat's liver and pancreas. Data are given as mean values \pm S.E. and they are expressed in ng/g wet weight. 3 in both groups

	Liver			Pancreas		
	Noradrenaline	Adrenaline	Dopamine	Noradrenaline	Adrenaline	Dopamine
3-month-old cats	457.8 \pm 61.5	1.6 \pm 0.2	10.6 \pm 1.9	254.3 \pm 47.5	4.4 \pm 1.7	34.6 \pm 16.0
Adult cats	183.0 \pm 24.7	4.1 \pm 1.5	3.5 \pm 0.6	251.2 \pm 77.0	3 \pm 0.5	27.5 \pm 13.6

blood samples for determination of plasma glucose concentration are obtained at intervals before, during and after the stimulation period (see Fig. 2). Atropine (0.5 mg/kg b.wt.) is given 15 min prior to the first control sample. Plasma glucose concentration is determined with the glucose-oxidase method.

In 3 expts the importance of the pancreatic sympathetic innervation for the glucose response to electrical stimulation was demonstrated in the following way. Both dorsal glands are removed. All sympathetic nerves were isolated around the celiac artery, few mm proximal to the bifurcation of the gastroduodenal artery. The nerves are then cut and the distal ends placed in bipolar platinum ring electrodes. These nerves consist of three distinct parts for the liver and the pancreas and when they all be called the hepatic and pancreatic sympathetic nerves. After recovery from surgery the nerves were stimulated with 10 Hz, 10 V and 1 ms for 10 min. Arterial blood samples for determination of serum haemoglobin (Hb), glucose (IRG) concentration and of plasma glucose concentration were withdrawn before and after the stimulation. The hepatic sympathetic nerves were then dissected free in the liver hilum as described above, cut, and placed in the platinum ring electrode. After further resting period of 15 min the same stimulation experiment was repeated. Hb and IRG were determined with radiocounterassay in previously described (Järnäck & Högl 1978).

Fluorescence microscopy. Liver and pancreas biopsies were taken from 3 anesthetized cats of two months age and from 4 anesthetized adult cats. The tissue pieces were frozen in propane-propylene mixture at the temperature of liquid nitrogen. After freeze-drying the preparations were processed for the fluorescence microscopical visualization of biogenic amines according to the Falck-Häflarp method (Falck et al. 1962; Falck 1964; for technical details, see Björklund, Falck & Owman 1972). The sections were mounted on microscope slides for fluorescence microscopy.

Chemical determinations. Noradrenaline, dopamine and adrenaline in liver and pancreatic tissue were determined by reversed-phase high-performance liquid chromatography using electrochemical detection (Hansson et al. 1979).

Pharmacological treatments. The adult cats were pretreated with the MAO-inhibitor mianserin (Normid Pfizer). One cat was given 10 mg/kg b.wt. of mianserin and the other 100 mg/kg b.wt. 3 h before it was sacrificed. Liver and pancreatic tissue were then processed for fluorescence microscopy as described above.

Four adult cats were treated with 6-hydroxydopamine (6-OH-DA) at a dose of 50 mg/kg b.wt. for 2 consecutive days. After another 6 days the same stimulation expt as described above was performed. After the end of each expt tissue specimens were obtained from the liver, pancreas and the right auricle of the heart for fluorescence

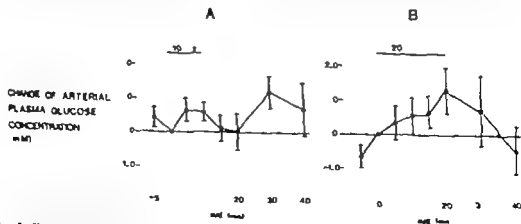


Fig. 2. Changes of arterial plasma glucose concentration in response to stimulation of the hepatic sympathetic nerves in 6-adrenalectomized cat given atropine (0.5 mg/kg b.wt.). Panel A shows the results from 9 expts in which the hepatic sympathetic nerves were stimulated during 10 min with 10 Hz, 10 V and 1 ms. Panel B shows the results from 4 expts in which the hepatic sympathetic nerves were stimulated during 20 min with 20 Hz, 20 V and 1 ms. Mean values \pm S.E. are given.

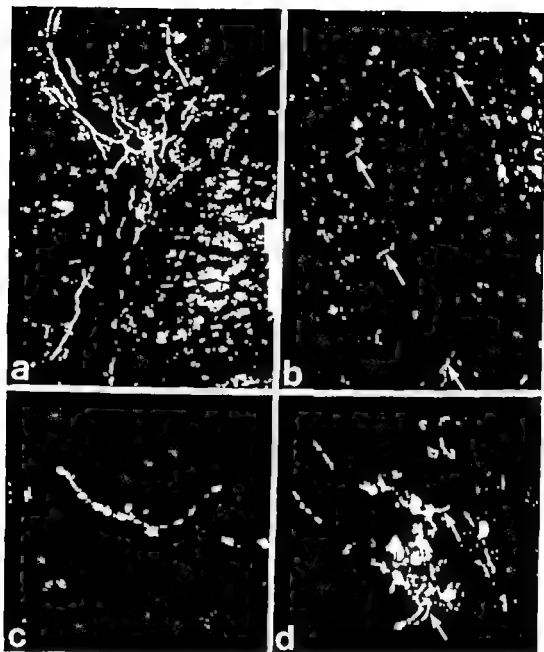


Fig 1 a-c are photomicrographs from the liver parenchyma of a two-month-old cat whereas d is taken from the endocrine pancreas of an adult cat. (a) represents the typical noradrenergic innervation to a major artery ($\times 120$). (b) shows the sparse parenchymal innervation. Noradrenaline-containing nerve fibres are pointed out with arrows ($\times 120$). (c) shows a noradrenaline-containing varicose nerve fibre running in close contact with the hepatocytes ($\times 180$). (d) shows noradrenaline-containing nerve fibres in an islet of Langerhans (marked with arrows). Note also the specific fluorescence of the endocrine cells ($\times 180$).

for at least 12 h before the expt. Anesthesia was induced with ether and maintained with chloralose (50 mg/kg) and urethane (100 mg/kg) given i.v. A tracheal cannula was inserted. The abdomen was opened with a midline incision and the adrenal glands were removed bilaterally. The right axillary artery was cannulated and connected to Statham P23AC transducers for recording of mean and pulsatile arterial blood pressure. Heart rate was recorded with a tachograph triggered by the systolic pressure wave. In all cats the hepatic sympathetic nerves were carefully dis-

sected free from the hepatic artery close to its entrance at the liver hilus. The nerve fibres were then cut and the distal ends placed in a bipolar platinum ring electrode connected to a Grass stimulator. All nerve fibres along the ga.roduodenal artery were cut in order to prevent any stimulation of the sympathetic nerves to the pancreas.

The animals were allowed to recover from surgery during 30-45 min. The hepatic sympathetic nerves were then stimulated for 10 min with 10 Hz, 10 V and 1 ms (6 cats) or for 20 min with 70 Hz, 70 V and 1 ms (4 cats). Arterial

concentration is about 250 ng/g wet weight i.e. a concentration similar to that found in the liver of the adult cat. Clearcut amounts of dopamine were also detected. There was no significant difference between young and adult cats with regard to the concentrations of the amines.

Effects of electrical nerve stimulation Fig. 2 shows how the arterial plasma glucose concentration was affected by electrical activation of the hepatic sympathetic nerves close to the liver hilus. If nervous elements along the gastroduodenal artery (described for the pancreas) were cut in these experiments. Panel A illustrates that arterial plasma glucose concentration increased slightly in response to 10 min period of stimulation with 10 Hz, 10 V and 1 ms (peak increment = 0.7 ± 0.3 mM, $P < 0.1$). Panel B shows that stimulation of the hepatic nerves with supramaximal intensity for 20 min (20 Hz, 70 V, 1 ms) caused a peak increment of plasma glucose concentration by 1.3 ± 0.6 mM ($P < 0.1$).

Fig. 3 illustrates that different effects on arterial plasma glucose, insulin (IRI) and glucagon (IRG) concentrations were observed when the hepatic sympathetic nerves were stimulated selectively (closed circles) or together with the pancreatic nerves (open circles) in the adrenalectomized cat. Stimulation of the hepatic and pancreatic sympathetic fibres thus evoked a clear-cut increment of arterial plasma glucose and IRG concentrations as well as a marked decrement of IRI concentration. When the electrode was placed on the hepatic nerves in the liver hilus (distal to the pancreatic nerve branches) electrical activation with the same characteristics caused no significant changes in arterial glucose, IRG or IRI concentrations.

No changes in systemic arterial blood pressure or in heart rate was observed in response to electrical stimulation of the hepatic sympathetic nerves.

Stimulation of the hepatic sympathetic nerves in adrenalectomized cat pretreated with 6-OH-DA did not change the arterial plasma glucose concentration.

DISCUSSION

The present fluorescence microscopical study of the feline liver has shown that the major portion of the sympathetic nerves which enter the liver is distributed to the intra-hepatic blood vessels. It was further demonstrated in contrast to previous studies (Ungvár & Donath 1969; Kyöskö 1978)

that there is a sparse adrenergic innervation of the liver parenchyma as well. The fact that the latter innervation has not been detected by the previous investigators might be due to technical difficulties in visualizing tiny adrenergic fibres. The existence of a sympathetic innervation to the cat liver parenchyma is in agreement with our earlier observations in the monkey and in man (Nobin et al. 1977, 1978) but the density of the parenchymal innervation in cat liver is clearly lower than that in man and monkey. This quantitative species difference was also reflected in the noradrenaline content of the liver which in the adult cat was 180 ng/g wet weight (Table 1) and in man 970 ng/g wet weight (Nobin et al. 1978). Marked species variations in the morphology of the sympathetic innervation of the liver have also been found in a comparative study on 7 different animals (Moghtezadeh et al., unpublished).

Our observation that the endocrine pancreas of the cat receives a dense sympathetic innervation is in agreement with Cegrell's original report (1968). This morphological finding is further supported by the demonstration of high concentrations of noradrenaline in pancreatic tissue (Table 1). There were also significant amounts of dopamine in the cat's pancreas which most likely emanate from the endocrine cells known to contain dopamine (Cegrell, Falck & Hellman 1964; Cegrell & Falck 1968; cf. Jacoby & Bryce 1978).

In 1977 Edwards reported that stimulation of the hepatic sympathetic innervation in the cat caused a prompt increment in the arterial plasma glucose concentration. Similarly it was found by one of us (Farbult 1975) that the hemorrhagic hyperglycemia in cats was markedly depressed by section of the hepatic sympathetic nerves. It was further demonstrated by Edwards that the rate at which glucose was released in response to nerve activation was graded in relation to the stimulation frequency and also depended on the amount of glycogen stored in the liver at the time of the experiment. With the same stimulation characteristics as used in the present study, Edwards found an increment in arterial plasma glucose concentration by 7–10 mM which far exceeds that of 1–2 mM observed in our experiments (Fig. 2). This discrepancy between the results might be explained on the assumption that in the two earlier studies the hepatic and pancreatic sympathetic nerves have been stimulated simultaneously. These pancreatic nerve branches emerge

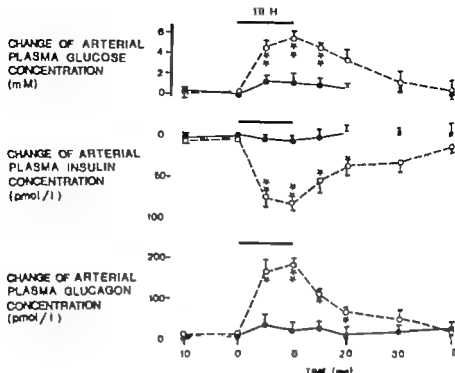


Fig 3 Changes of arterial plasma glucose, glucagon and insulin concentrations in response to stimulation (10 Hz, 10 V, 1 ms) of the hepatic and pancreatic sympathetic nerves (O---O) or to stimulation of the hepatic sympathetic nerves selectively (●---●). The expts. were performed on 3 adrenalectomized cats and 60 min elapsed between the end of the first stimulation and the beginning of the second stimulation. Atropine (0.5 mg/kg b.wt.) was given prior to both stimulation periods. Mean \pm S.E. are given. Asterisks indicate a statistical difference between the two curves: $P < 0.05$, $P < 0.01$.

microscopy and chemical determination of noradrenaline, adrenaline and dopamine.

Statistical methods. All data are expressed as a mean value \pm S.E. using the Student's *t*-test for the statistical analysis.

RESULTS

Fluorescence microscopy. Fluorescent varicose nerve fibres were found in the interlobular spaces associated with blood vessels of both portal and hepatic origin (Fig. 1a). The density of these vessel fibres was comparable in the 2-month-old kittens and in the adult cats. In the liver parenchyma scattered varicose sympathetic nerve fibres contacting individual liver cells could be demonstrated (Fig. 1b,c). Although the number of fibres were few they were evenly distributed throughout the parenchyma. It was observed that the liver parenchyma of the 2-month-old kittens seemed to have a more dense adrenergic innervation than that of the adult cats.

After pretreatment with the MAO-inhibitor the

intensity of the fluorescent nerve structures increased, but no significant increment in the number of nerve fibres was detected.

Adrenergic sympathetic fibres were also observed in the islets of pancreas (Fig. 1d). These nerve fibres were localized in apposition to the endocrine cells and their distribution was similar in the young and adult cats. After pretreatment with 6-OH-DA the liver and endocrine pancreas were completely devoid of fluorescent nerve fibres. Even in the right heart auricle, a richly innervated sympathetic tissue, all sympathetic catecholamine-containing nerves had disappeared.

Chemical determinations. The results are summarized in Table 1. The analysis showed that liver tissue from both young and adult cats contained significant amounts of noradrenaline, the concentration of which was about twice as high in young as in adult cats. After pretreatment with 6-OH-DA the concentration of noradrenaline in the liver fell below the detection level.

Table 1 also illustrates the content of the 3 catecholamines in the pancreas. The noradrenaline

- ROSENGREN E. The distribution of the sympathetic nervous system in mammalian liver tissue—a chemical and fluorescence histochemical study. *Cell Tissue Res.* (in press).
- JOBIN A., BAUMGARTEN H. & FALCK, B. IN: INGEMANSSON S., MOGHIMZADEH E. & ROSENGREN E. 1978. Organization of the sympathetic innervation of liver tissue from monkey and man. *Cell Tissue Res.* 195: 371–380.
- JOBIN A., FALCK, B., INGEMANSSON S., JARHULT J. & ROSENGREN E. 1977. Organization and function of the sympathetic innervation of human liver. *Acta Physiol Scand. Suppl.* 452: 103–106.
- LYDOUX J., BRUNSMANN M. J. A., JEAN-RENAUD B. & GIRARDIER, L. 1979. Alpha-sympathetic control of glucose output of mouse liver per fixed μ min. *Amer. J. Physiol.* 236: E323–E327.
- SHIMAZU T. & AMAKAWA A. 1968a. Regulation of glycogen metabolism in liver by the autonomic nervous system. II. Neural control of glycogenolytic enzymes. *Biochim. Biophys. Acta* 165: 335–348.
- SHIMAZU T. & AMAKAWA A. 1968b. Regulation of glycogen metabolism in liver by the autonomic nervous system. III. Differential effects of sympathetic nerve stimulation and of catecholamines on liver phosphorylase. *Biochim. Biophys. Acta* 165: 349–356.
- SHIMAZU T. & FUJIMOTO T. 1971. Regulation of glycogen metabolism biosynthesis. *Biochim. Biophys. Acta* 252: 18–77.
- UNGVARY G. & DONATH, T. 1969. On the monoaminergic innervation of the liver. *Acta Anat. (Basel)* 72: 446–459.

from the sympathetic plexus in close connection to the liver hilus and very often they are completely hidden by the portal vein and the common bile duct. When these pancreatic nerves were stimulated concomitantly to the hepatic nerves in our expts the magnitude of the glucose response approached that reported by Edwards (Fig. 3 open circles). Support for our contention that the direct adrenergic innervation of the feline liver is responsible for only a minor part of the total glycemic response to the sympathetic stimulation comes from a recent study by Lutt & Wong (1978). They showed that arterial plasma glucose concentration rose by about 3 mM in response to hepatic nerve activation in cats provided the gastroduodenal artery (with its adjacent pancreatic nerve branches) had been ligated. Also in anesthetized man stimulation of the hepatic sympathetic nerves increased arterial plasma glucose concentration by about 2 mM (Nobin et al 1977 Järhult et al 1979).

We conclude that in the cat there is a dense sympathetic innervation both to the pancreatic endocrine cells and to the hepatic vasculature whereas the sympathetic innervation to the liver parenchyma is sparse. The pronounced hyperglycemic effect of activation of the sympathetic nervous system in the cat is mediated mainly via an influence on the release of glucagon and insulin from the pancreas and only to a minor extent via a direct neurogenic effect on glucose release from the hepatocytes.

This study was supported by grants from the Swedish Medical Research Council (04X-00036, 04X-0071 and 04X-110) the Medical Faculty University of Lund and from Thorsten and Svea Segerfalks Foundation.

REFERENCES

- BJÖRKLUND A, FALCK B & ÖWMAN C 1977 Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In *Methods of investigation and diagnostic endocrinology* (ed S A Berson) vol 1 pp 318-368. North-Holland, Publ Comp.
- CEGRELL L & FALCK B 1968 The development of monoamine containing cell in guinea-pig pancreas. *Acta Physiol Scand Suppl* 314: 4-31.
- CEGRELL L, FALCK B & HELLMAN B 1964 Monoaminergic mechanisms in the endocrine pancreas. In *Structure and metabolism of the pancreatic islets* (ed by S E Brodin, B Hellman and H Knutson) pp 479-435. Pergamon Press, Oxford.
- EDWARDS A V 1971 The glycogenolytic response: stimulation of the splanchnic nerves in adrenalectomized calves, sheep, dogs, cats and pigs. *J Physiol (Lond)* 13: 741-759.
- EDWARDS A V 1977a The sensitivity of the hepatic glycogenolytic mechanism to stimulation of the splanchnic nerves. *J Physiol (Lond)* 220: 315-334.
- EDWARDS A V 1977b The hyperglycaemic response to stimulation of the hepatic sympathetic innervation in adrenalectomized cats and dogs. *J Physiol (Lond)* 220: 697-710.
- EDWARDS A V & SILVER M 1972 Comparison of the hyperglycaemic and glycogenolytic responses to catecholamines with those to stimulation of the hepatic sympathetic innervation in the dog. *J Physiol (Lond)* 23: 571-593.
- FALCK B 1967 Observations on the possible cellular localization of monoamines by a fluorescence method. *Acta Physiol Scand* 96 (Suppl 197).
- FALCK B, HILLARP N Å, THIELE G & TÖRÖ A 1962 Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J Biochem Cytolchem* 10: 348-354.
- FALCK B, INGEMANSSON S & NOBIN A 1975 The adrenergic innervation of the human liver. *Acta Endocrinol (Abstr)* Suppl 19: 181.
- GREENWAY C V & STARK R D 1971 Hepatic vasculature. *Physiol Rev* 51: 23-65.
- HANSSON C, AGRUP G, RÖRSMAN H, ROSENQREN A M & ROSENQREN E 1979 Analysis of cysteinyl-dopa, dopamine, noradrenaline and adrenaline in serum and urine using high-performance liquid chromatography and electrochemical detection. *J Chromatography* 166: 7-21.
- JACOBY J H & BRYCE G F 1978 Monoamine modulation of pancreatic endocrine secretion. *Gen Pharmacol* 9: 411-419.
- JÄRHULT J 1975 Role of the sympatho-adrenal system in haemorrhagic hyperglycaemia. *Acta Physiol Scand* 93: 25-33.
- JÄRHULT J, FALCK B, INGEMANSSON S & NOBIN A 1979 The functional importance of hepatic nerves to the liver and endocrine pancreas. *Ann Surg* 189: 96-100.
- JÄRHULT J & HOLST J J 1978 Reflex adrenergic control of endocrine pancreas evoked by unloading of carotid baroreceptors in cats. *Acta Physiol Scand* 104: 188-202.
- KYÖSOLA K 1978 Comparative formaldehyde-induced and glyoxylic acid-induced fluorescence histochemical studies on the intrinsic adrenergic innervation of the intestine and the liver of normal and vagotomized cats. *Acta Histochem* 6: 183-199.
- LAUTT W W 1979 Neural activation of alpha-adrenoreceptors in glucose mobilization from liver. *Can J Physiol Pharmacol* 57: 1037-1039.
- LAUTT W W & WONG C 1978 Hepatic glucose balance in response to direct stimulation of sympathetic nerves in the intact liver of cat. *Can J Physiol Pharmacol* 56: 1022-1028.
- MOGHIMZADEH E, JÄRHULT J, NOBIN A &

- ROSENGREN E. The distribution of the sympathetic nervous system in mammalian liver tissue—a chemical and fluorescence histochemical study. *Cell Tiss Res* in press.
- ROBIN A, BAUMGARTEN H G, FALCK B, INGEMANSSON S, MOGHIMZADEH L & ROSENGREN E. 1978. Organization of the sympathetic innervation in liver tissue from monkey and man. *Cell Tiss Res* 195: 371–380.
- ROBIN A, FALCK B, INGEMANSSON S, JARHULT J & ROSENGREN E. 1977. Organization and function of the sympathetic innervation of human liver. *Acta Physiol Scand*, Suppl. 452: 103–106.
- RYDOUX J, BRUNSMANN M J A, JEAN RENAUD B & GIRARDIER L. 1979. Alpha-sympathetic control of glucose output of mouse liver per branch in situ. *Amer J Physiol* 236: E323–E327.
- SHIMAZU T & AMAKAWA A. 1968. Regulation of glycogen metabolism in liver by the autonomic nervous system. II. Neural control of glycogenolytic enzymes. *Biochim Biophys Acta* 165: 334–348.
- SHIMAZU T & AMAKAWA A. 1968b. Regulation of glycogen metabolism in liver by the autonomic nervous system. III. Differential effect of sympathetic nerve stimulation and of catecholamines on liver phosphorylase. *Biochim Biophys Acta* 165: 349–356.
- SHIMAZU T & FUJIMOTO T. 1971. Regulation of glycogen metabolism biosynthesis. *Biochim Biophys Acta* 252: 18–27.
- UNGVARY M & DONATI L. 1969. On the monosynaptic innervation of the liver. *Acta Anat (Basel)* 72: 446–499.

from the sympathetic plexus in close connection to the liver hilus and very often they are completely hidden by the portal vein and the common bile duct. When these pancreatic nerves were stimulated concomitantly to the hepatic nerves in our expts the magnitude of the glucose response approached that reported by Edwards (Fig. 3 open circles). Support for our contention that the direct adrenergic innervation of the feline liver is responsible for only a minor part of the total glycemic response to the sympathetic stimulation comes from a recent study by Lutt & Wong (1978). They showed that arterial plasma glucose concentration rose by about 3 mM in response to hepatic nerve activation in cats provided the gastroduodenal artery (with its adjacent pancreatic nerve branches) had been ligated. Also in anesthetized man stimulation of the hepatic sympathetic nerves increased arterial plasma glucose concentration by about 2 mM (Nobin et al 1977; Järhult et al 1979).

We conclude that in the cat there is a dense sympathetic innervation both to the pancreatic endocrine cells and to the hepatic vasculature whereas the sympathetic innervation to the liver parenchyma is sparse. The pronounced hyperglycemic effect of activation of the sympathetic nervous system in the cat is mediated mainly via an influence on the release of glucagon and insulin from the pancreas and only to a minor extent via a direct neurogenic effect on glucose release from the hepatocytes.

This study was supported by grants from the Swedish Medical Research Council (04X-00056, 04X-0071 and 04X-2210), the Medical Faculty, University of Lund and from Thorsten and Sves Segerfalks Foundation.

REFERENCES

- BJÖRKLUND A, FALCK B & ÖWMAN C 1977 Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In *Methods of investigation and diagnostic endocrinology* (ed S A Berson) vol 1 pp 318-368. North-Holland, Publ. Comp.
- CEGRELL L & FALCK B 1968 The development of monoamine containing cells in guinea-pig pancreas. *Acta Physiol Scand Suppl* 314: 4-31.
- CEGRELL L, FALCK B & HELLMAN B 1964 Monoaminergic mechanisms in the endocrine pancreas. In *Structure and metabolism of the pancreatic islet* (ed by S E Brodin, B Hellman and H Krut von) pp 479-435. Pergamon Press, Oxford.
- EDWARDS A V 1971 The glycogenolytic response: stimulation of the splanchnic nerves in adrenalectomized calves, sheep, dogs, cats and pigs. *J Physiol (Lond)* 213: 741-759.
- EDWARDS A V 1972a The sensitivity of the hepatic glycogenolytic mechanism to stimulation of the splanchnic nerves. *J Physiol (Lond)* 220: 315-334.
- EDWARDS A V 1972b The hyperglycaemic response to stimulation of the hepatic sympathetic innervation in adrenalectomized cats and dogs. *J Physiol (Lond)* 220: 697-710.
- EDWARDS A V & SILVER M 1977 Comparison of the hyperglycaemic and glycogenolytic responses to catecholamines with those to stimulation of the hepatic sympathetic innervation in the dog. *J Physiol (Lond)* 263: 571-593.
- FALCK B 1962 Observations on the possibilities of cellular localization of monoamines by a fluorescence method. *Acta Physiol Scand* 56 (Suppl. 197).
- FALCK B, HILLARP N Å, THIEME G & TÖRÖ A 1966 Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J Biochem. Cytochem* 10: 348-354.
- FALCK B, INGEMANSSON S, NOBIN A & ROSENGREN E 1975 The adrenergic innervation of the human liver. *Acta Endocrinol (Kbb)* (Suppl. 19) p. 181.
- GREENWAY C V & STARK R D 1971 Hepatic vascular bed. *Physiol Rev* 51: 3-65.
- HANSSON C, ÅGRUP G, RÖRSMAN H, ROSENGREN A M & ROSENGREN E 1979 Analysis of cysteinylglycine, dopamine, noradrenaline and adrenaline in serum and urine using high-performance liquid chromatography and electrochemical detection. *J Chromatography* 16: 7-22.
- JACOBY J H & BRYCE G F 1978 Monoaminergic modulation of pancreatic endocrine secretion. *Gen Pharmacol* 9: 411-419.
- JÄRHULT J 1975 Role of the sympatho-adrenal system in haemorrhagic hyperglycaemia. *Acta Physiol Scand* 93: 3-33.
- JÄRHULT J, FALCK B, INGEMANSSON S & NOBIN A 1979 The functional importance of sympathetic nerves to the liver and endocrine pancreas. *Ann Surg* 189: 96-100.
- JÄRHULT J & HOLST J J 1978 Reflex adrenergic control of endocrine pancreas evoked by unloading of carotid baroreceptors in cats. *Acta Physiol Scand* 104: 188-200.
- KYÖSOLA M 1978 Comparative formaldehyde-induced and glyoxylic acid-induced fluorescence histochemical studies on the intrinsic adrenergic innervation of the intestine and the liver of normal and vagotomized cats. *Acta Histochem* 62: 188-199.
- LAUTT W W 1979 Neural activation of splanchnic renoreceptors in glucose mobilization from liver. *Can J Physiol Pharmacol* 57: 1037-1039.
- LAUTT W W & WONG C 1978 Hepatic glucose balance in response to direct stimulation of sympathetic nerve in the intact liver of cat. *Can J Physiol Pharmacol* 56: 1022-1028.
- MOGHIMZADEH E, JÄRHULT J, NOBIN A &

The effect of chronic nicotine and withdrawal on intra-neuronal dynamics of acetylcholine and related enzymes in a preganglionic neuron system of the rat

DAHLSTRÖM A, BOÖJ S, HIRWALL P-O & LARSSON P-A

Institute of Neurobiology, University of Göteborg, Sweden

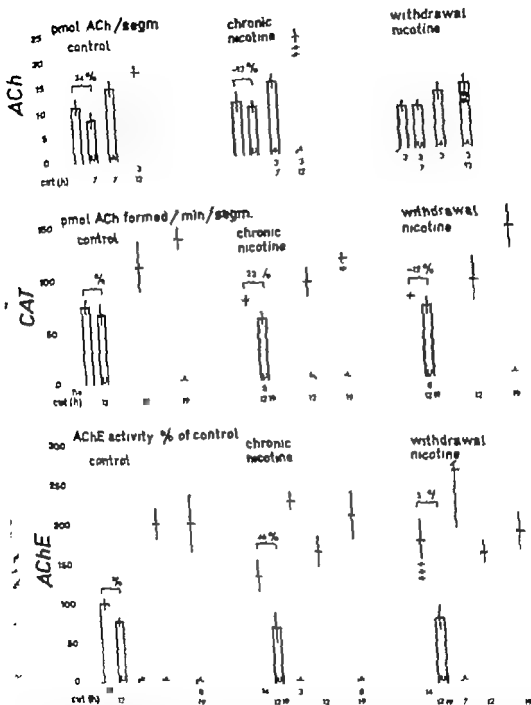
DAHLSTRÖM A, BOÖJ S, HIRWALL P-O & LARSSON P-A. The effect of chronic nicotine and withdrawal on intra-neuronal dynamics of acetylcholine and related enzymes in a preganglionic neuron system of the rat. *Acta Physiol Scand* 1980; 110: 13-20. Received 2 Nov 1979. ISSN 0001-6772. Institute of Neurobiology, University of Göteborg, Sweden.

The effect of chronic nicotine treatment given in the drinking water for 8-10 weeks in doses equivalent to that of a heavy cigarette smoker and of withdrawal 2 days on acetylcholine (ACh), choline acetyltransferase (CAT) and AChE-esterase (AChE) activities in the preganglionic cervical nerve and the superior cervical ganglion (SCG) of rats, were studied. Control rats were housed and handled as the nicotine rats. After cutting the preganglionic nerve 7-19 h before dissection, ACh, CAT and AChE accumulated in the nerve part proximal to the cut (relative to the nerve cell bodies in the spinal cord). A clearance of these substances was observed in the nerve distal to the cut. This indicates that all 3 substances are transported proximo-distally in this preganglionic cholinergic nerve. In the SCG, ACh was decreased already by 7 h (to about 60%), while CAT and AChE-activities were lowered to 60% and 80% respectively at 19 h after cutting the nerve. Chronic nicotine treatment caused an increased ACh accumulation (by about 35%) and a decreased CAT accumulation (by about 20%) in the cut nerve, while the ganglionic level of all 3 substances were essentially unchanged. Withdrawal of nicotine for 2 days prior to the final experiment caused reduced ACh-accumulation (by about 30%) in the nerve and not in the SCG, while the CAT accumulation. The AChE-activity of intact nerve was markedly increased to about 175% of control and the transportable fraction of AChE (clearance distal to the cut) was about twice as large as in control. In the SCG withdrawal caused marked changes in the ACh content, which was decreased to 62% of control. CAT-activity was increased to 117% of control while AChE was unchanged. Our hypothesis is that the withdrawal from chronic nicotine treatment and in particular withdrawal of nicotine may cause marked alterations in the activity of the preganglionic neuron. This may induce changes in the neuronal transport of the 3 substances and an increased turnover of ACh in the nerve terminal of the SCG after withdrawal of nicotine.

Key words: Chronic nicotine withdrawal, acetylcholine, cholinergic enzymes, preganglionic nerve, cervical superior ganglion, intra-neuronal transport.

Nicotine in small doses has a stimulating effect on autonomic ganglia. The drug activates the so-called nicotinic cholinergic receptors on ganglion cells and this effect may be abolished by the ganglionic blocking agent hexamethonium (see discussion in Völle & Koelle 1970). However, comparatively low doses of nicotine may also have a local action on adrenergic and possibly also on cholinergic nerve endings, thus causing a release of the transmitters (cf. Trendelenburg 1965; Westfall & Bravstedt 1972).

Apart from these effects on cholinergic nerve endings and receptors, nicotine may also have direct or indirect effects on intracellular events e.g. on the intra-axonal transport. Earlier we have reported effects of acute non-toxic doses of nicotine on acetylcholine esterase (AChE) levels in intact and crushed sciatic nerve of rat. In the present paper we have studied the influence of chronic nicotine treatment and withdrawal on the acetylcholine (ACh), AChE and choline acetyltransferase (CAT)



The effect of chronic nicotine treatment and 7 days withdrawal on acetylcholine (ACh) content (upper part), choline acetyltransferase (CAT) activity (middle part) and acetylcholine esterase (AChE) activity (lower part) in rat cervical sympathetic ganglionic nerve. The values are given per segment intact, A or U (4-5 mm lengths, Fig. 2). AChE-values are expressed in % of control intact nerve for each experimental occasion. n = number of observations, and cut (h) = hour after cutting the nerve. The differences between intact segments and U-segments are indicated in the figure. Vertical bars indicate S.E.M. * = $P < 0.05$ ** = $P < 0.01$ ΔΔ = $P < 0.005$ compared to corresponding segments in the control group. Δ = $P < 0.01$ compared to the nicotine group.

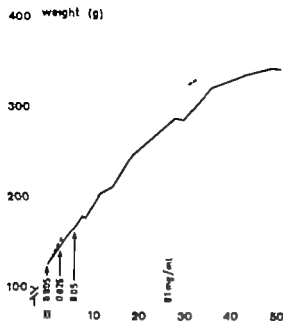


Fig. 1 Illustration of increase in body weight of rats during chronic nicotine treatment (—) compared to controls (---). The arrows indicate changes in nicotine concentrations in the drinking water during a period of 50 days. Abscissa: days of treatment.

levels in the cervical preganglionic neuron system of the rat. The dose of nicotine given was chosen to be equivalent to that of a heavy cigarette smoker.

MATERIAL AND METHODS

Nicotine treatment. Male albino rats (Sprague Dawley) having an initial weight of 130 g and a final weight of about 340 g were used. The rats were divided into 3 groups: 2 of which received nicotine (Nicotine base, Leo) in the drinking water (tap water) for up to 10 weeks, starting at a concentration of 5 µg/ml, reaching 100 µg/ml finally (see Fig. 1). The third group (controls) were given plain water. All rats were kept 5 in each cage and housed together at 25°C on a constant 12 h lighting schedule. The water intake (per cage of 5 rats) and the body weight of all groups of animals were recorded at regular intervals. The nicotine solutions prepared from nicotine base were studied regularly by spectrofluorimetric assays of Millipore-filtered samples to control the stability of the nicotine.

At the end of the nicotine period, each rat drank on the average 27 ml (± 4 S.D.) of a 0.1 mg/ml nicotine solution/24 h, corresponding to an intake of 2.7 mg of nicotine per rat or about 8 mg/kg/day. Control rats drank about 33 ml/day/rat. One of the nicotine groups was made abstinent by excluding nicotine from their drinking water days before the final experiment (withdrawal group).

Operative procedure. Under ether anaesthesia the cervical preganglionic nerve bilaterally was cut with a fine pair of scissors just under the m. sternomastoideus (Fig. 2). Some rats in each group were used unoperated. Seven

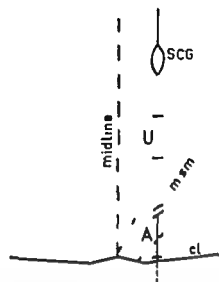


Fig. 2 Schematic illustration of the cervical preganglionic nerve and the sympathetic cervical ganglion (SCG). The nerve was cut under m. sternomastoideus (m). The segment A (4–5 mm) proximal to the cut (relative to the cell bodies located in the spinal cord) and segment (5–10 mm) distal to the cut (i.e. closer to the SCG) were dissected out 7 or 19 hours after operation. In operated rats a 17–20 mm length of nerve from 5 mm caudal to the SCG was dissected and cut into 4–5 mm segments for analysis. cl = clavicle.

1 or 19 h after operation the rats were re-anaesthetized and the superior cervical ganglion (SCG) and the preganglionic nerve were dissected out. The nerves were placed on an ice-chilled glassplate and cut into 4–5 mm long segments relative to the cut as indicated in Fig. 2 with the aid of an operation microscope. Intact nerves were dissected out from 5 mm caudal to the SCG or 17–20 mm caudally and cut into 4–5 mm segments for analysis.

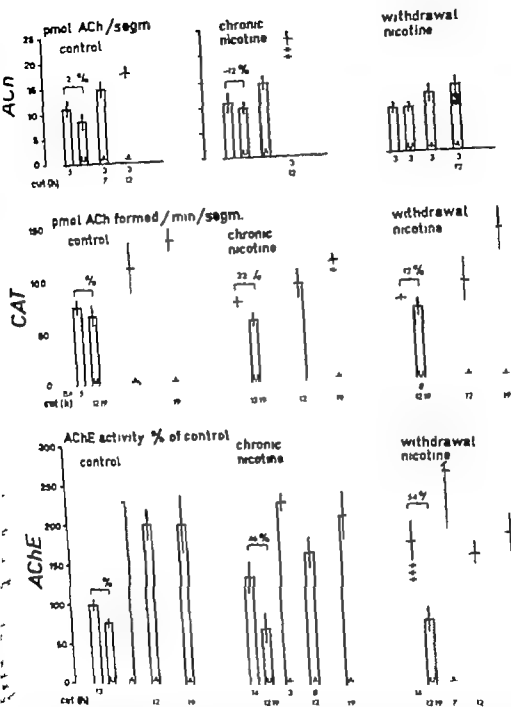
ACh was assayed biologically on a guinea pig liver preparation according to Blaber & Cuthbert (1961). Segments were assayed, but 10–30 nerve segments had to be pooled for each ACh assay. The activity of the samples in the gut could be abolished by incubating the samples with purified AChE (Worthington Biochemical Corporation, Freehold, N.J.) or by treatment of the gut with atropine (10 M). The measured activity (thru AChE) will be referred to as ACh in the following.

Enzymes were assayed radiochemically using the methods of Fonnum (1969) (AChE) and Glover & Gert (1971) (CAT). Single ganglia or 6–10 nerve segments (4–mm long) were pooled for each assay.

RESULTS

Preganglionic nerve

The ACh-content in intact nerve was 11 ± 1.5 pmol per 4–5 mm segment in the control group and 11 ± 1.9 pmol in the nicotine group. The withdrawal



3 The effect of chronic nicotine treatment and 2 days withdrawal on acetylcholine (ACh) content (upper part), choline acetyltransferase (CAT) activity (middle part) and acetylcholine esterase (AChE) activity (lower part) in rat cervical sympathetic ganglionic nerve. The values are given per segment intact, A or U (4-5 mm length) (Fig. 1). ChE-values are expressed in % of control intact nerve for each experimental occasion. n = number of observations, cut (h) = hours after cutting the nerve. The differences between intact segments and U-segments are indicated in the figure. Vertical bars indicate S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ compared to corresponding segment of the control group. **** $P < 0.01$ compared to the nicotine group.

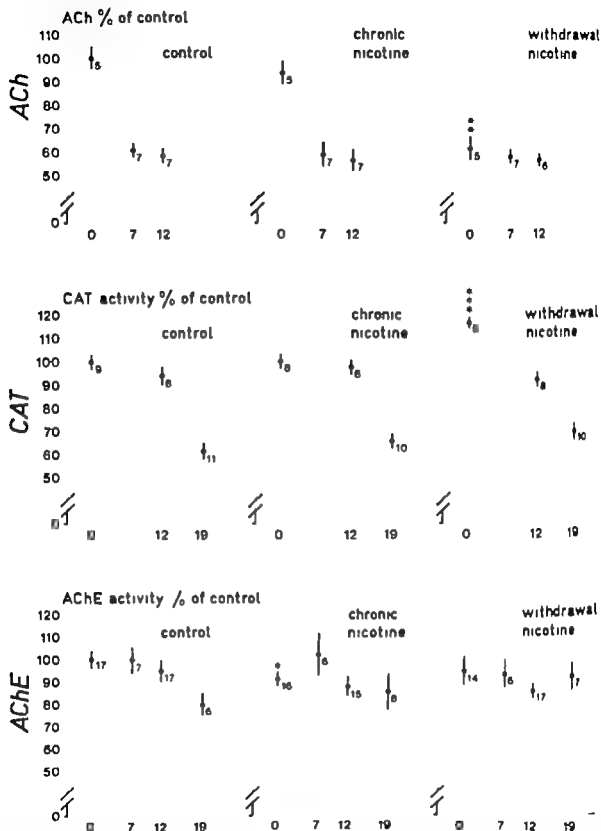


Fig. 4 The effect of chronic nicotine treatment and 2 days withdrawal on acetylcholine (ACh) content and the activities of choline acetyltransferase (CAT) and acetylcholinesterase (AChE) in the rat superior cervical ganglion. The values are expressed in % of intact control ganglia. Small figures indicate number of observations. Vertical bars indicate \pm S.E.M. $n=1$ 0.05 $P < 0.005$ compared to the control group.

up had somewhat lower ACh content (8.4 ± 1.4 nmol/segment). In all 3 groups the ACh content in the T-segment increased with time after cutting. This increase was higher than control in the nicotine group at 1 h ($P < 0.01$) and lower than in the other groups and control after withdrawal ($P < 0.01$) (Fig. 3). In the U-segment (analysed in an attempt to disclose the 'transportable fraction' of Häggendal et al. 1973) small insignificant decrease was observed in the control and nicotine groups, but not in the withdrawal group.

The CAT-activity of intact nerve was about 75 pmol of ACh formed/min/4.5 mm segment in all 3 groups. An increase with time in the A segment was observed, but this increase was at 19 h smaller in nicotine group than control (Fig. 3). The U-segment 5–10 mm distal (i.e. 'proximal') to the cut (see Fig. 1) appeared to contain less enzyme activity in the intact segments in each group, but the difference was not statistically significant. After chronic nicotine treatment the accumulation of T at 12 and 19 h was decreased, but normalized to after withdrawal.

The AChE-activity in intact control nerve varied between 910 and 4165 pmol of ACh split per min/4.5 mm segment at the different experimental occasions. The reason for this variance is unknown. Therefore, the AChE results are expressed in % of intact control nerve at each experimental occasion. In each experiment the control value was about 0.7 ± 0.1 in the chronic nicotine group, intact nerve segments contained $136 \pm 20.3\%$ and in the withdrawal group the increase was even larger $18 \pm 23.8\%$ ($P < 0.005$). The clearance of AChE in the U-segment distal to the cut was 44% after chronic withdrawal as compared to 22% in control and 46% in the nicotine group. The activity of the T-segments was by 7 h increased (between 230 and 30% of control) in nicotine but no further increase in enzyme activity appeared to occur in this segment between 7 and 19 h (Fig. 3). This is possibly due to accumulation of AChE in more proximal, not analysed, segments. No changes were seen after control or withdrawal.

The ACh content of control ganglia was 273 ± 9.2 pmol of ACh ($100 \pm 3.7\%$). After denervation the level was reduced to 61.2% and to 19% at 7 and 12 h respectively. In the chronic nicotine group the intact ganglia contained 94% of

control ganglia and following denervation the levels decreased to 59% and 46% respectively. Intact ganglia of the withdrawal group had only 6.7% of control ($P < 0.005$) but the level after denervation were similar to those in the denervated ganglia of the two other groups (Fig. 4).

The CAT-activity of control ganglia was 79 ± 3 pmol of ACh formed/min/gg ($100 \pm 3\%$). At 1 h after denervation the activity was 94% of control and had decreased to 61% at 19 h (Fig. 4 middle). The same pattern was seen in the nicotine group. In the withdrawal group however the level of enzyme activity in the intact ganglia was 117% of normal ($P < 0.005$) but following denervation the same decrease occurred as in the other groups.

The AChE-activity was estimated on two occasions. 100% on the first occasion was 49.541 ± 4.095 pmol of ACh split/min/gg ($n = 10$). On the second occasion 100% corresponded to 117.109 ± 10.19 pmol of ACh split/min/gg ($n = 7$). In the control group a small decrease was observed at 19 h after denervation (80% $P < 0.01$). In the nicotine group the AChE-activity of intact ganglia was 97% ($P < 0.05$) but no decrease was observed after denervation. In the withdrawal group the ganglia had about the same activity as the control group (Fig. 4 bottom).

DISCUSSION

The ACh content of control preganglionic nerve and ganglia found in the present study as well as the reactions to nerve sectioning correspond well with those of previous study (Häggendal et al. 1973). The CAT-activity of control ganglia (79 pmol $60 = 47.5$ nmol of ACh formed/gg/h) also compares well with previous results (e.g. Debrer et al. 1977: 55 nmol of ACh formed/gg/h). The AChE-activity of the present study (117 and 49 nmol of ACh split/gg/min) is of the same magnitude as previously reported (e.g. McLennan 1954: about 84 nmol/gg/min and Klingman et al. 1968: 28.8 nmol/gg/min). We have not found values of activity of the cholinergic enzymes in the preganglionic nerve in the literature.

All 3 substances appeared to undergo proximo-distal transport in the preganglionic nerve. This is indicated by the gradual increase in segment A with time after axotomy (Fig. 3) and also by the decrease in amount in segment U (distal to the axotomy) 7–19 h after cutting, suggesting a clear

ance due to continued intra axonal transport in the distal nerve stump (cf. Ekström & Emmelin 1971). For AChE the increase in segment A was maximal at 7 h. This may be due to continued accumulation in segments further proximally (not assayed) or to a reversed transport of AChE in a retrograde direction known to occur for AChE in ligated sciatic nerves (e.g. Bisby & Bulger 1977). The rates of proximo-distal transport of the various substances cannot be calculated with certainty due to large scatter of the SEM rendering calculation on the transportable fractions of ACh, CAT and AChF difficult. However, the increase in ACh proximal to the cut is of the same order (160%) as in the previous study (Häggendal et al. 1973, 145% above control) which may (with a transportable fraction of 5-6, Häggendal et al. 1973) indicate a transport rate for ACh in the control group of between 117-170 mm/day. It is clear that also CAT and AChF undergo considerable transport in this nerve; this will be further investigated.

The effect of axotomy on the ganglia shows that ACh was decreased already 7 h after operation while CAT and AChF activities were significantly decreased at 19 h. Since most of the ACh and CAT and a considerable part of the AChE in the SCG is located in nerve terminals of the preganglionic cholinergic neurons (cf. Dhar 1958, Gromadzki & Koelle 1965, Joki et al. 1971, Tuček 1978) this was an expected finding confirming previous observations (e.g. Banister & Scrase 1950, Erdinko & Härkönen 1965, Häggendal et al. 1973).

The nicotine dose given was chosen to give serum levels similar to that of a heavy smoker. According to Wenzel & Azmeh (1970) who also gave nicotine chronically with the drinking water a dose of 4 mg/kg/day is equivalent to that of a heavy smoker. In this study we gave about 8 mg/kg/day which is a high dose but not giving any toxic symptoms.

The result from the chronic nicotine group differed from the control values on 3 points: (1) The ACh-accumulation at 17 h was higher than control in preganglionic nerve; (2) the CAT activity in the same nerve was slightly reduced at 19 h postoperatively; and (3) the AChE-activity in unoperated preganglionic nerve was somewhat elevated (Fig. 3). In the ganglia no differences were observed. The chronic pharmacological stimulation of the nicotine receptors in the ganglia during the treatment period thus had no marked effects on the cholinergic sub-

stances in the ganglia. The increased 17 h accumulation of ACh in the preganglionic nerve is due to the behaviour of the cholinergic enzymes in nerve. CAT-accumulation was somewhat decreased (not increased) while AChE activity was increased (not decreased). Nicotine has been reported to release ACh from synaptic vesicles (Chou 1970). If nicotine had caused a release of ACh from axonal organelles, this would have caused decrease of ACh in the nerve, since ACh was then become available for AChF-degradation, as an increase as in this study. The reason for increased ACh accumulation is not known at present but may depend on the activity of the neuron.

The most dramatic changes occurred after withdrawal of nicotine for 7 days. In the nerve the ACh accumulation was much reduced to near sub-normal levels, the CAT accumulation was normalized, and the AChE-activity of intact nerve even more increased to 178% of control value (Fig. 3). In the ganglia ACh content was decreased to about 62% of control while the CAT-activity was increased to about 117% of control (Fig. 4). These observations may hypothetically be explained in the following way. After withdrawal of nicotine the artificial stimulation of the nicotine receptors cease. Due to reflex and local feedback mechanisms the release of ACh from the nerve terminals of the ganglion must increase. This can be accomplished by an increased impulse activity in the preganglionic neuron or an increase in the amount of ACh released per nerve impulse or a combination of both. The decrease in ACh-content in the ganglia after withdrawal clearly suggests release of ACh to an extent exceeding the rate of ACh synthesis. The synthesis on the other hand is not likely to be subnormal, since CAT-activity was increased (Fig. 4). AChE-activity was unchanged. The observations that CAT activity was decreased after withdrawal suggest that the impulse activity of the neuron was raised. It is known from other autonomic cholinergic neurons that the rate of activity of the neuron may determine the activity of CAT in its nerve terminals (cf. Ekström 1971). Since there is no evidence for the presence of activators or inhibitors of CAT (Erdinko 1970, Tuček & Brimppoin 1975) the increased enzyme activity in rat SCG after nicotine withdrawal suggests that the supply of enzyme from the soma by means of axonal transport is increased. The rate of transport of CAT in the preganglionic nerve may be

order of 40 mm/d (if the transportable fraction is $\times 10^4$), i.e. 10 times higher than in the motor nerves of rat and rabbit (cf. Tuček 1975 Saunders *et al.* 1973), but only 3 times faster than reported in the vagus nerve of rabbit (Frizell *et al.* 1970 Fonnium *et al.* 1973). Since the length of the preganglionic neuron may be about 20–30 mm the increased CAT-activity in the ganglia after days of chronic withdrawal may well be caused by an increased somatal synthesis of the enzyme due to increased activity and subsequently increased axonal transport to the nerve endings.

The results of the present study show that chronic administration of nicotine in a dose which mimics that obtained by heavy smokers causes biochemical changes in an autonomic cholinergic neuron. These changes may well be secondary to an altered functional state of the neuron, caused by nicotine treatment and in particular by withdrawal. The clear changes in ganglionic ACh and CAT after days of nicotine withdrawal may be related to the well-known abstinence syndrome

supported by grants from the Swedish Tobacco Company (nos. 7310, 7415, 7519, 7611, 7713) and from the Swedish Medical Research Council (grants 04X, 2207, 04P-0173). I thank the Swedish Laro Pharmaceutical Co. Halmstad (Sweden) for generous supply of nicotine.

REFERENCES

- ANISTER, J. & SCRASE, M. 1970 Acetylcholine synthesis in normal and denervated sympathetic ganglia of the cat. *J. Physiol.* 111, 437–444.
- SBY, N. A. & BULGER, V. I. 1977 Reversal of axonal transport at nerve crush. *J. Neurochem.* 29, 313–320.
- ABER, L. C. & CUTHBERT, A. W. 1961 A sensitive method for the assay of acetylcholine. *J. Pharm. Pharmacol.* 13, 425–446.
- CHOU, C. Y., LONG, J. P., POTREPKA, R. & SPIRATT, I. L. 1979 The ability of various nicotine agonists to release acetylcholine from synaptic vesicles. *Arch. Int. Pharmacodyn.* 187, 88–96.
- HALL, S. K. 1954 Cholinesterase in decentralized and autonomic sympathetic ganglia. *J. Physiol. (Lond.)* 104, 77.
- ABDEL, M. D., MYTILINEOU, C. & BLACK, I. B. 1977 Target organ regulation of sympathetic neuron development. *Brain Research* 123, 301–310.
- STRÖM, J. 1978 Acetylcholine synthesis and its dependence on nervous activity. *Experientia* 34, 1247–1253.
- STRÖM, J. & EMVELIN, N. 1971 Movement of choline acetyltransferase in axons disconnected from their cell bodies. *J. Physiol.* 216, 247–256.
- ERÄNKÖ, O. & HÄRKÖNEN, M. 1963 Effect of axon division on the distribution of noradrenaline and acetylcholinesterase in sympathetic neurons of the rat. *Acta Physiol. Scand.* 63, 411–412.
- FONNUM, F. 1969 Radiochemical micro-assay for the determination of choline acetyltransferase and acetylcholinesterase activities. *Biochem. J.* 115, 465–472.
- FONNUM, F. 1970 Studies of choline acetyltransferase with particular reference to a subcellular localization. Norwegian Defence Research Establishment, Report No. 58.
- FONNUM, F., FRIZELL, M. & SJÖSTRAND, J. 1973 Transport, turnover and distribution of choline acetyltransferase and acetylcholinesterase in the vagus and hypoglossal nerves of the rabbit. *J. Neurochem.* 19, 1109–1120.
- FRIZELL, M., HASSELGREN, P.-O. & SJÖSTRAND, J. 1970 Axoplasmic transport of acetylcholinesterase and choline acetyltransferase in the vagus and hypoglossal nerves of the rabbit. *Exp. Brain Res.* 10, 526–531.
- GLOVER, V. & GREEN, D. P. L. 1977 A simple quick microassay for choline acetyltransferase. *J. Neurochem.* 19, 465–466.
- GROSIADZKI, C. G. & KOELLE, G. B. 1965 The effect of votomy on the acetylcholinesterase of the superior cervical ganglion of the cat. *Biochem. Pharmacol.* 14, 1745–1754.
- HÄGGENDAL, C. J., DAHLSTRÖM, A. B. & SAUNDERS, N. R. 1973 Axonal transport and acetylcholine in rat preganglionic neurones. *Brain Research* 58, 484–499.
- JABLECKI, C. & BRIMUON, S. 1974 Axoplasmic transport of choline acetyltransferase activity in mice: effect of age and neurotoxicity. *J. Neurochem.* 25, 581–593.
- JOO, E., LEVER, J. D., IVENS, C., MOTTRAM, D. R. & PRESLEY, R. 1971 A fine structural and electron histochemical study of axon terminals in the rat superior cervical ganglion after acute and chronic preganglionic denervation. *J. Anat.* 110, 181–189.
- KLINGMAN, G. S., KLINGMAN, J. B. & POLISZCZUK, A. 1968 Acetyl- and pseudocholinesterase activities in sympathetic ganglia of rats. *J. Neurochem.* 15, 1121–1130.
- MCLENNAN, H. 1954 Acetylcholine metabolism of normal and axotomized ganglia. *J. Physiol.* 104, 113–116.
- SAUNDERS, N. R., DZIEGIELEWSKA, K., HÄGGENDAL, C. J. & DAHLSTRÖM, A. B. 1973 Slow accumulation of choline acetyltransferase in crushed vagus nerves of the rat. *J. Neurobiol.* 4, 95–103.
- TRENDELENBURG, U. 1965 Nicotine and the peripheral autonomic nervous system. In *Tobacco alkaloids and related compounds* (ed. U. S. Ecker), vol. 4, pp. 167–178. Pergamon Press, London.
- TUČEK, S. 1975 Transport of choline acetyltransferase and acetylcholinesterase in the central stump and isolated segments of peripheral nerve. *Brain Research* 86, 259–270.
- TUČEK, S. 1978 Acetylcholine synthesis in neurones. Chapman and Hall, London.

- VOLLE R & KOELLE O B 1970 Ganglionic stimulating and blocking agents. In *The pharmacological basis of therapeutics* (ed. L. S. Goodman & A. Gilman) pp. 566-574. Macmillan Publ. Co.
- WENTZEL D G & AZMEH N 1970 Chronically administered nicotine and the blood pressure of normotensive and renal hypertensive rats. *Arch Int Pharmacodyn* 187: 367-376.
- WESTFALL T C & BRASTED M 1971 The mechanism of action of nicotine on adrenergic nerves in the perfused guinea-pig heart. *J Pharmacol Ther* 18: 409-417.

Compensatory increase of responses to nerve stimulation in the partially denervated rat urinary bladder

RIGEN EKSTRÖM and MATS ELMÉR

Department of Physiology and Biophysics, University of Lund, Sweden

EKSTRÖM J & ELMÉR M. Compensatory increase of responses to nerve stimulation of the partially denervated rat urinary bladder. *Acta Physiol Scand* 1980 110: 1-29. Received 7 Nov 1979. ISSN 0001-6777. Department of Physiology and Biophysics, University of Lund, Sweden.

The rat urinary bladder was deprived of half of its innervation by removing the pelvic ganglion on one side. The motor responses of such partially denervated bladder to stimulation of the pelvic nerve on the other side were examined 1 week, 1 month and 2 months postoperatively. On all three occasions the increase in pressure of the operated bladder was larger than that of the control bladder; the enlargement was most marked 2 months after operation. The responses were further enlarged by eserine and markedly reduced by atropine. The present results combined with those of previous investigations showing rapid recovery in the activity of the acetylcholine-forming enzyme from reduced level and transient supersensitivity to chemical stimuli after unilateral removal of the pelvic ganglion, suggest that the enlarged responses to nerve stimulation 1 week postoperatively are mainly due to sensitization, while those observed at the later stages are due to collateral sprouting from the cholinergic nerve fibres of the intact pelvic nerve.

Key words: Collateral sprouting, urinary bladder, rat.

Morphological and physiological investigations now show that the urinary bladder of the rat is well supplied with cholinergic nerve fibres which travel not only in the pelvic nerve but also in the hypogastric nerve (Alm & Elmér 1975; Ekström & Elmér 1977; see Elmér 1978). Most of the fibres of the hypogastric nerve pass through the pelvic ganglion on their way to the bladder (Langworthy 1965; Urison, Fletcher & Bradley 1973). Bilateral removal of this ganglion causes the acetylcholinesterase-positive nerves in the bladder to disappear (Alm & Elmér 1975) and the activity of the acetylcholine-synthesizing enzyme, choline acetyltransferase, to fall to 5% or less of the normal value (Ekström & Elmér 1977). Unilateral removal of the ganglion is followed by rapid recovery of choline acetyltransferase activity from 38% of the normal activity on the 3rd postoperative day to 86% on the 25th postoperative day (Barros, Ekström & Mann 1978, 1979). This recovery in enzyme activity has been suggested to depend on increased motor impulse traffic in the remaining nerves and on out-

growth of these nerves in the partially denervated bladder (see Ekström 1978). The finding of supersensitivity of the urinary bladder to chemical stimuli 1 week but not 4 weeks after such a partial denervation seems to be in accordance with these suggestions (Ekström, Elmér & Barros 1978).

In the present study the electrically evoked motor responses of the urinary bladder on stimulation of the intact pelvic nerve have been examined on different occasions following removal of the pelvic ganglion on the opposite side. Since adrenergic nerve fibres reach the bladder via the same routes as the cholinergic ones (Alm & Elmér 1975) the motor effects of both types of nerves had to be considered. However, in contrast to the cholinergic innervation, the adrenergic innervation of the detrusor muscle is sparse (Alm & Elmér 1975).

METHODS

A total of 60 adult male rats of a Sprague-Dawley strain bred at the Department of Physiology were used. Under ether anaesthesia the abdomen was opened and with the aid

Table 1 Bladder weights in operated rats 1 week, 1 month and 2 months after unilateral removal of pelvic ganglion and in unoperated control rats

Values are mean \pm S.E. Number of rats is indicated in brackets

	1 week		1 month		months	
	Weight (mg)	Operated/unoperated %	Weight (mg)	Operated/unoperated %	Weight (mg)	Operated/unoperated %
Operated	105.6 \pm 7.8 (13)		99.0 \pm 3.4 (8)		95.8 \pm 4.4 (9)	
Unoperated	92.9 \pm 3.1 (13)	114.3 \pm 6	85.2 \pm 4.4 (8)	117.4 \pm 5.3	89.3 \pm 4.5 (9)	108.7 \pm 5

 $P < 0.001$ < 0.02 > 0.1 when the bladder weight of the operated rat is compared with that of the control litterate

of a dissecting microscope the right pelvic ganglion with its nerve filaments close to the bladder was removed. The wound was then sutured. One week, one month or two months after surgery the operated animals and their litter mate controls were anesthetized with chloralose (100 mg/kg) through a femoral venous cannula after induction with ether.

Intravesical pressure recording. To record the pressure in situ the bladder was exposed and the ureters were ligated. A glass cannula was inserted into the bladder through an incision in the urethra and 0.25 ml of physiological saline solution was introduced. The pressure developed by the detrusor muscle was recorded by means of a transducer and a polygraph. The resting pressure was about 5 mmHg.

Nerve stimulation. Using a bipolar electrode the pelvic nerve (including the fibres from the hypogastric nerve which pass through the pelvic ganglion) was stimulated electrically after section just proximal to the pelvic ganglion. In some of the control rats the pelvic nerves of both sides were stimulated simultaneously. Grass stimulators supplied with stimulus isolation units giving rectangular pulses with a duration of 2 ms, either as single shocks or with a frequency of 0.5–30 Hz and 100 Hz and of supramaximal intensity (10V) were used. The stimulation period was 15 s. The stimulation sequence was repeated after injection of eserine sulphate, then after an additional injection of atropine sulphate and eventually after the injection of dihydroergotamine methanesulphonate.

In the operated bladders the region of the prostatic gland where the ganglion is situated was inspected; no nerve strands were found.

At the end of the experiment the bladder was removed, cleaned and weighed.

The operated and unoperated litter mates used for comparison were always tested on the same occasion.

Statistics. Student's *t*-test was used paired comparisons were made between the operated rat and its unoperated litter mate. The 5% level of probability was considered significant.

RESULTS

There was no significant difference in body weights between the operated rats and their controls in any

of the three series. In the 1 week series the operated rats weighed (mean \pm S.E.) 354 \pm 11 g; the 13 unoperated rats 354 \pm 10 g. In the 1-month series the 8 operated and 8 control rats weighed 361 \pm 15 g and 345 \pm 12 g respectively and in the 2-month series the 9 operated and 9 control rats weighed 357 \pm 17 g and 368 \pm 15 g respectively.

In the groups of rats examined 1 week and 1 month postoperatively the partially denervated bladders were 14 and 17% heavier than those of

mmHg Unoperated

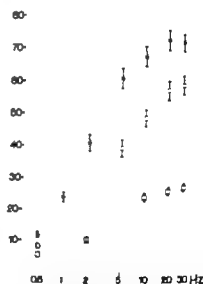


Fig. 1 Control bladders. The mean pressure (and S.E.) of the urinary bladder of all the 30 control rats in response to various stimulation frequencies of the pelvic nerve. The difference between the response obtained before drug administration and that after eserine or atropine (in the presence of eserine) was significant ($P < 0.001$) at all frequencies examined. The symbols are as follows: \bullet before drug administration, \blacksquare after eserine, \square after atropine.

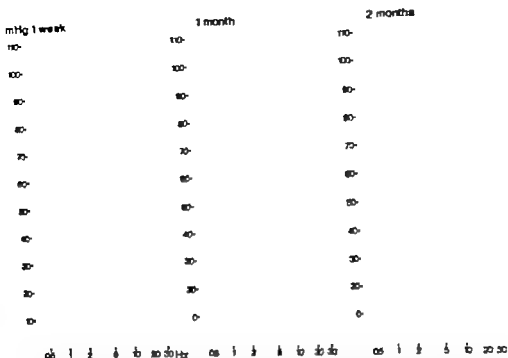


Fig. 2. Partially denervated bladders. The mean pressure increase of the urinary bladder in response to stimulation of the intact pelvic nerve: 1 week (13 rats), 1 month (8 rats) and 2 months (9 rats) after partial denervation. The difference between the response obtained before drug administration and that after eserine or atropine (in the presence of eserine) was significant ($P < 0.05$ or less), except for the result obtained before and after eserine administration at 20 and 30 Hz 2 months postoperatively. Symbols as in Fig. 1.

control (Table 1). In the 2-month series the difference of 9 mmHg between operated and control bladders was not large enough to be significant.

Motor responses of the unoperated bladders. Fig. 1 shows the mean (and S.E.) increase in intra-vesical pressure of the 30 control bladders developed in response to electrical stimulation of the pelvic nerve at frequencies varying between 0.5 and 30 Hz before and after administration of eserine and of atropine. In Fig. 3 the pressure rise obtained in response to a single electrical shock applied to the nerve is shown (open columns); in this figure the responses are presented separately for each group of observations.

The mean increase in pressure in all the controls was 59 ± 0.4 mmHg as response to a single shock and 55.5 ± 0.8 mmHg in response to 70 Hz; a plateau was reached at 20–30 Hz.

In the presence of eserine (0.1 mg/kg), the response to a single shock, 61 ± 0.4 mmHg, was not significantly larger than that observed before the administration of the drug; however at 0.5–30 Hz

the pressure responses after the administration of the cholinesterase inhibitor were larger than before. The responses were particularly enlarged at low frequencies: e.g. at 1 and 5 Hz the percentage gain was 85–90% whereas at 30 Hz it was about 30%.

In the presence of atropine (1 mg/kg) the effect of eserine on the pressure responses was abolished, and the responses both to single shocks and to the frequencies 0.5–30 Hz were found to be significantly lower than those obtained before the eserine administration; they were diminished by 40–55%. The percentage reduction was least at the single shock level where the increase in pressure was 3.6 ± 0.1 mmHg.

In order to reveal a possible contribution by the adrenergic nerves to the persisting pressure increase after administration of atropine the stimulation sequence was repeated in the presence of the α -adrenoceptor blocking drug *alhydrotol* (mg/kg); 100 Hz was used since such a high frequency had previously been found necessary to evoke an α -adrenoceptor mediated contrac-

Table 1 Bladder weights in operated rats 1 week, 1 month and 2 months after unilateral removal of pelvic ganglion and in unoperated control rats

Values are mean \pm S.E. Number of rats is indicated in brackets

	1 week		1 month		months	
	Weight (mg)	Operated/unoperated**	Weight (mg)	Operated/unoperated**	Weight (mg)	Operated/unoperated**
Operated	105.6 \pm 8 (13)	114.3 \pm 6	99.0 \pm 3.4 (8)	117.4 \pm 5.3	95.8 \pm 4.4 (9)	108.7 \pm 5.1
Unoperated	97.9 \pm 3.1 (13)		85.2 \pm 4.4 (8)		89.3 \pm 4.5 (9)	

 $P < 0.001$, < 0.05 , > 0.1 when the bladder weight of the operated rat is compared with that of the control littermate

of a dissecting microscope the right pelvic ganglion with its nerve filaments close to the bladder was removed. The wound was then sutured. One week, one month or two months after surgery the operated animals and their littermate controls were anesthetized with chloralose (100 mg/kg) through a femoral venous cannula after induction with ether.

Intravesical pressure recording. To record the pressure in situ the bladder was exposed and the ureters were ligated. A glass cannula was inserted into the bladder through an incision in the urethra and 0.5 ml of physiological saline solution was introduced. The pressure developed by the detrusor muscle was recorded by means of a transducer and a polygraph. The resting pressure was about 5 mmHg.

Nerve stimulation. Using a bipolar electrode the pelvic nerve (including the fibres from the hypogastric nerve which pass through the pelvic ganglion) was stimulated electrically after section just proximal to the pelvic ganglion. In some of the control rats the pelvic nerves of both sides were stimulated simultaneously. Grass stimulators supplied with stimulus isolation units giving rectangular pulses with a duration of 1 ms either as single shocks or with a frequency of 0.5–30 Hz and 100 Hz and of supramaximal intensity (10V) were used. The stimulation period was 15 s. The stimulation sequence was repeated after injection of eserine sulphate, then after an additional injection of atropine sulphate and eventually after the injection of dihydroergotamine methanesulphonate.

In the operated bladders the region of the prostatic gland where the ganglion is situated was inspected; no nerve strands were found.

At the end of the experiment the bladder was removed, cleaned and weighed.

The operated and unoperated littermates used for comparison were always tested on the same occasion.

Statistics. Student's *t* test was used; paired comparisons were made between the operated rat and its unoperated littermate. The 0.05 level of probability was considered significant.

RESULTS

There was no significant difference in body weights between the operated rats and their controls in any

of the three series. In the 1 week series the 13 operated rats weighed (mean \pm S.E.) 354 ± 11 g; the 13 unoperated rats 354 ± 10 g. In the 1 month series the 8 operated and 8 control rats weighed 361 ± 15 g and 345 ± 17 g respectively, and in the 2 month series the 9 operated and 9 control rats weighed 357 ± 17 g and 368 ± 15 g respectively.

In the groups of rats examined 1 week and 2 months postoperatively the partially denervated bladders were 14 and 17% heavier than their

mmHg Unoperated



Fig. 1. Control bladders. The mean pressure (mean \pm S.E.) of the urinary bladder of all the 30 control rats in response to various stimulation frequencies of the pelvic nerve. The difference between the response obtained before drug administration and that after eserine or atropine (in the presence of eserine) was significant ($P < 0.001$) at all frequencies examined. The symbol C as follows: C before drug administration, E after eserine, A after atropine.

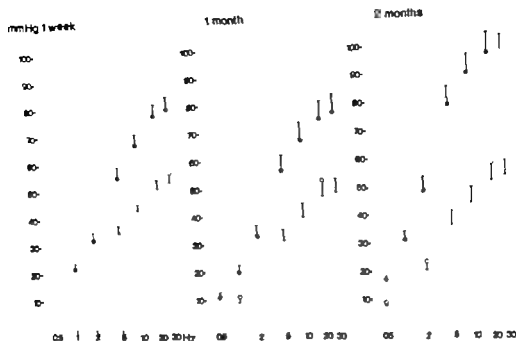


Fig. 4. The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve 1 week (13 rats), 1 month (11 rats) and 2 months (9 rats) after partial denervation (filled circles) and of control (open circles). The difference between operated and control bladders was significant ($P < 0.02$ or less) at all frequencies.

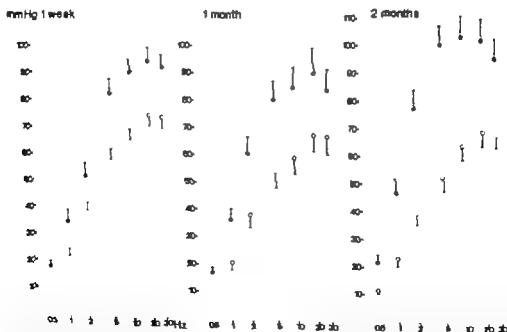


Fig. 5. Eserine. The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve 1 week (13 rats), 1 month (8 rats) and 2 months (9 rats) after partial denervation (filled circles) and of controls (open circles) in the presence of eserine. The difference between operated and control bladders was significant ($P < 0.02$ or less) at all frequencies.

mmHg

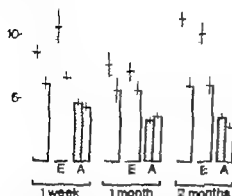


Fig. 3 Single shocks. The mean pressure increase (and S.E.) of the urinary bladder in response to single shock stimulation applied to the intact pelvic nerve 1 week (13 rats), 1 month (8 rats) and 2 months (9 rats) after partial denervation (hatched columns) and of corresponding controls (open columns) before and after the administration of eserine (E) and after atropine (A). Comparisons are made between operated and control bladders and when the difference is significant it is indicated as follows: $P < 0.05$ $P < 0.01$ $P < 0.001$.

tion of the bladder (Elmér 1975a). In Fig. 8 it can be seen, however, that at a frequency of 30 Hz the pressure increase was already somewhat lower after the administration of dihydroergotamine than before, at 100 Hz the difference was more obvious. In the 18 rats examined the pressure increases before and after dihydroergotamine at 30 Hz were 26.1 ± 1.8 and 23.4 ± 1.9 mmHg. At the frequency of 100 Hz the figures before and after the α blocker were 23.7 ± 1 and 18.6 ± 2.0 mmHg, respectively.

The rat urinary bladder is known to be supplied with β adrenoceptors mediating relaxation (Elmér 1974, 1975a, Ekström 1979). In this type of study, however, a pressure fall below the resting value was very seldomly found to follow on the pressure rise. In those cases where it was observed it did not exceed 1 mmHg.

Motor responses of the operated bladders. In Fig. 1 the mean pressure increases obtained in response to stimulation of the intact pelvic nerve at a frequency of 0.5–30 Hz are summarized at different times after partial denervation, before and after eserine and after atropine. These curves are also shown in Figs. 4–6 where comparisons are made with corresponding controls. In these cases S.E. is indicated. In Fig. 7 the effect of dihydroergotamine on the pressure of the operated bladders is shown

after administration of eserine and atropine. Responses to single shock stimulations (last columns) are shown in Fig. 3.

In response to a single shock (Fig. 3) the 1- and 2-month postoperative bladders developed significantly larger pressure rises than their controls, while in the 1-month series the enlargement observed only tended to be significant ($0.05 < P < 0.1$). The enlargements expressed as percentages were 47, 37 and 85, respectively. In all three series the responses of the operated bladders to 0.5–30 Hz were significantly larger than those of the controls (Fig. 4). The largest increases were seen at the low frequencies, the smallest at the high. In the 1-week series the pressure responses of the operated bladders were enlarged by 77–41%, in the 1-month series by 79–41% and in the 2-month series by 117–61%. In the 2-month series the pressure increases found at stimulations of 70 Hz in 4 operated animals were found to be 117, 90, 92 and 75% of those found after combined stimulation of the pelvic nerve on both sides of the 4 corresponding control rats. These percentage figures should be compared with those obtained in the control rats when the pressure increase caused by stimulation of the left pelvic nerve is expressed as a percentage of that obtained when both left and right nerves were stimulated simultaneously; they were 63, 76, 68 and 55.

In the presence of eserine the responses of the operated bladders were also larger than those of the controls both to single shocks and repetitive stimulations. At the level of single shocks the response was enlarged by 61, 29 and 68% 1 week, 1 month and 2 months postoperatively (Fig. 3). At 0.5–30 Hz the responses were enlarged by 50–33% in the 1-week series, by 78–25% in the 1-month series and by 107–40% in the 2-month series (Fig. 5). The response to single shocks in the operated animals like the controls was not potentiated by eserine, neither was the response potentiated at high frequencies in the operated animals of the 2-month series.

In the presence of atropine the rise in pressure in response to single shocks was about the same in operated and unoperated bladders in the 1-week and 1-month series (Fig. 3). In contrast the operated bladders in the 2-month series responded with a pressure rise 24% larger than the controls—a difference which was significant. In the 1-week series the responses of the operated bladders were

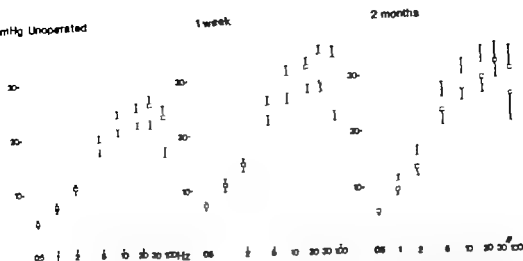


Fig. 8. The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve in the absence of eserine and atropine (\square), and after additional administration of dihydroergotamine (\circ) in 18 control bladders, bladders partially denervated 1 week in advance and 5 bladders partially denervated 2 months in advance. When significant the difference before and after dihydroergotamine is indicated as follows: $P < 0.05$, $P < 0.01$, $P < 0.001$.

were found to be larger than those in the control bladders when examined at various times after partial denervation. The most marked differences in pressure responses between operated and control bladders were found in the 2-month series which covered the longest time of observation. As judged from the experiments in which eserine and atropine were used, the major part of the enlarged responses can be attributed to the activation of cholinergic receptors. It is well-known in several species, including the rat, that not all of the rise in pressure developed in response to nerve stimulation is atropine sensitive (Langley & Anderson 1895; Carpenter & Robin 1967; Chesher 1970; Carpenter 1977; see also Elmér 1978). A major portion of the persisting pressure rise at high frequencies of pelvic nerve stimulation is found to be due to activation of α -adrenoceptors in the control bladders at 30 Hz, and in the bladders partially denervated 1 and 2 months in advance at 100 Hz, in contrast in the bladders partially denervated for one week α -adrenoceptors were activated already at 10 Hz.

The outcome of the present experiments combined with that of previous investigations indicates that the intact pelvic nerve gradually extends its field of innervation in the partially denervated bladder. The enlarged motor responses to stimulation of the pelvic nerve are maintained, while the initially developed sensitization to the injection of

methacholine and also to noradrenaline disappears (Elmér, Elmér & Banno 1978); the activity of choline acetyltransferase increases (Banno, Elmér & Mann 1978, 1979); this enzyme is confined to the cholinergic nerves of the bladder (Ekström 1975; Elmér & Elmér 1977).

The enlarged motor responses found one week after partial denervation are probably explained mainly by the fact that acetylcholine released on stimulation of the intact pelvic nerve acts on sensitized muscle cells, as seems also to be the case for noradrenaline. It should be mentioned that a selective adrenergic nerve destruction by 6-hydroxydopamine is not followed by an unspecific sensitization towards methacholine (Elmér 1979). By the 6th postoperative day the activity of choline acetyltransferase has increased by 17% from 58% of the activity of the controls found on the 3rd day, an observation which may signal beginning of collateral nerve sprouting and reflect also an increase in the traffic of motor impulses in the intact nerve. One month postoperatively sensitization does not seem to contribute to the enlarged motor responses, since the bladder at this stage was not found to respond with greater pressure rise than its control after administration of methacholine or noradrenaline. At this time of observation the activity of choline acetyltransferase has increased further being 86% of the activity found in control bladders.

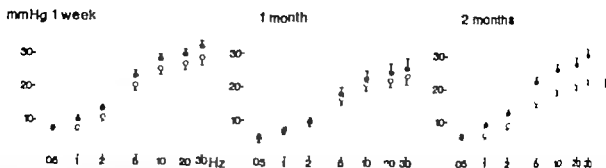


Fig. 6 Atropine. The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve 1 week (13 rats), 1 month (8 rats) and 2 months (9 rats) after partial denervation (filled circles) and of controls (open circles) in the presence of atropine (and of eserine). In the series of 1 week and of 2 months the difference between operated and control bladders was significant ($P < 0.05$ or less) at all frequencies. In the series of 1 month significance could not be demonstrated.

significantly larger than those of the controls at 0.5–30 Hz, the percentage enlargement being 30–17% (Fig. 6). In the 1-month series there was no difference in the responses between operated and unoperated bladders at these frequencies. However, in the 2-month series significantly larger responses were again found in the operated bladders compared with those of controls at all frequencies (0.5–30 Hz), the responses being enlarged by 48–29% and as earlier being most marked at the low frequencies.

When comparing the responses of operated bladders in the presence of atropine (and eserine) with those obtained after administration of *dihydroergotamine* (see Fig. 8) it was found in the 1-week series that at a frequency of 10 Hz activation of α adrenoceptors already contributed to the response; the rise in pressure was 8–14% less after the α blocker at 10–30 Hz and at 100 Hz it was 77% less. Conversely in the series of 1 and 2 months the responses tended to be larger after the α -blockade than before except at high stimulation frequencies.

In the 1-month series not shown in Fig. 8, the response at 30 and 100 Hz was 5 and 17% less than before, and at 100 Hz in the 2-month series 14% less.

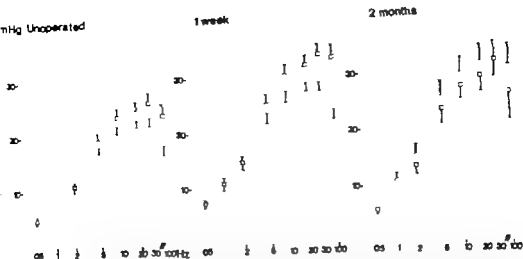
In the three series the responses obtained in the presence of *dihydroergotamine* were significantly larger in the operated than in the control bladders at frequencies from 0.5 to 30 Hz with a few exceptions (see Fig. 7). 1 week after operation the responses were enlarged by 44–14%, 1 month after by 45–11% and 2 months after operation by 57%. The mean pressure rise in the operated bladders to single shocks was 18, 43 and 79% in controls 1 week, 1 month and 2 months post-operatively. It was only in the 2-month series that the enlargement was significant. As in the control bladders a pressure decrease below resting level occurred rarely.

DISCUSSION

In the operated bladders the pressure rises evoked by stimulation of the intact pelvic nerve were



Fig. 7 *Dihydroergotamine*. The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve 1 week (10 rats), 1 month (3 rats) and 2 months (5 rats) after partial denervation (filled circles) and of controls (open circles) in the presence of *dihydroergotamine* (and of eserine and atropine). The difference between operated and control bladders was significant ($P < 0.05$ or less) at all frequencies except at 0.5 Hz in the 1-month series.



8 The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve in the presence of eserine and atropine (\square), and after additional administration of dihydroergotamine (\circ) in 11 control bladders, bladders partially denervated 1 week in advance and 3 bladders partially denervated 2 months in advance. When significant the difference before and after dihydroergotamine is indicated as follows: $P < 0.01$, $P < 0.01$, $P < 0.001$.

and to be larger than those in the control bladders when examined at various times after partial denervation. The most marked differences in pressure responses between operated and control bladders are found in the 2-month series which covered the longest time of observation. As judged from the experiments in which eserine and atropine were used, the major part of the enlarged responses can be attributed to the activation of cholinergic receptors. It is well-known in several species, including the rat, that not all of the rise in pressure developed in response to nerve stimulation is atropine sensitive (Langley & Anderson 1895; Carpenter & Rubin 1967; Chesler 1970; Carpenter 1977; see also Elmér 1978). A minor portion of the persisting pressure rise at high frequencies of pelvic nerve stimulation as found to be due to activation of α -adrenoceptors in the control bladders at 30 Hz, and in the bladders partially denervated 1 and 2 months in advance at 100 Hz, in contrast in the bladders partially denervated for one week α -adrenoceptors are activated already at 30 Hz.

The outcome of the present experiments, combined with that of previous investigations, indicates that the intact pelvic nerve gradually extends its field of innervation in the partially denervated bladder. The enlarged motor responses to stimulation of the pelvic nerve are maintained, while the initially developed sensitization to the injection of

methacholine and also to noradrenaline disappears (Ekström, Elmér & Banno 1978). The activity of choline acetyltransferase increases (Banno, Ekström & Klarm 1978, 1979); this enzyme is confined to the cholinergic nerves of the bladder (Ekström 1975; Ekström & Elmér 1977).

The enlarged motor responses found one week after partial denervation are probably explained mainly by the fact that acetylcholine released on stimulation of the intact pelvic nerve acts on sensitized muscle cells, as seems also to be the case for noradrenaline. It should be mentioned that a selective adrenergic nerve destruction by 6-hydroxydopamine is not followed by an unspecific sensitization towards methacholine (Ekström 1979). By the 6th postoperative day the activity of choline acetyltransferase has increased by 17% from 58% of the activity of the controls found on the 3rd day, an observation which may signal beginning of collateral nerve sprouting and reflect also an increase in the traffic of motor impulses in the intact nerve. One month postoperatively sensitization does not seem to contribute to the enlarged motor responses since the bladder at this stage was not found to respond with greater pressure rise than its control after administration of methacholine or noradrenaline; at this time of observation the activity of choline acetyltransferase has increased further being 86% of the activity found in control bladders.

Two months postoperatively the motor responses were most enlarged e.g. stimulation of the intact pelvic nerve led to a pressure increase that was about the same as that produced in control bladders by simultaneous stimulation of the two pelvic nerves. In normally innervated bladders of the rat a functional overlap of the two pelvic nerves of about 20% has been found at 15–20 Hz (Carpenter & Rubin 1967; Elmér 1975b). Using this percentage figure it can be calculated that the intact pelvic nerve in the operated bladder produced a mean pressure rise that was 5% larger than that obtained in the control bladders if the two nerves had been stimulated at the same time at 20 Hz in all the controls of the 7 month series while in the 1 month series the operated bladder produced a mean pressure rise that was 10% less than that of the controls. The activity of choline acetyltransferase did not increase beyond the 1-month stage. This may suggest that the sprouting process is complete 1 month postoperatively while during the following period of time changes occurred in the neuro-effector region that increased the efficiency of the transmission of nerve impulses.

The present investigation like the previous ones (Banns Ekström & Mann 1978, 1979; Ekström Elmér & Banns 1978) shows that in the period that follows operation the bladder gains in weight. The gain is lost later. In this study no significant difference in the weight between operated and control bladders was found at the latest observation time. The bladder weight increase after partial denervation and that after complete denervation (Ekström & Elmér 1977) is probably due to similar causes i.e. distension of the bladder due to difficulties in bladder-emptying. Thus the disappearance of the slight gain in weight in the series of 2 months may be taken as a further sign of a successful reinnervation.

Unfortunately bladders partially denervated by removal of the pelvic ganglion have not been investigated as to changes in the distribution of acetylcholinesterase positive nerves. However in such a denervated bladder of the rat an outgrowth of adrenergic nerves from the intact half of the bladder as judged by fluorescent technique has been found to have occurred when examined 6–9 weeks postoperatively (Aim & Elmér 1979). In contrast to the cholinergic the adrenergic nerves do not distribute bilaterally (Carpenter & Rubin 1967; Elmér 1975b). The bilateral distribution of the cholinergic nerves

is probably a favourable condition for sprouting (appears from studies on sympathetic ganglia (Nay & Thompson 1957) and skeletal muscles (Edds 1953; Brown & Irons 1978)). Organically studied that sprouting is favoured when generating and intact nerves intermingle with other. In partially denervated skeletal muscle the rat collateral sprouting was demonstrated histologically 4–5 days postoperatively it was particularly abundant during the third week. Connected with the endplates the collaterals were found to mature and early in the second postoperative month they resembled normal nerve fibres. A regain in function was found to occur likewise.

The motor responses of the rat urinary bladder have been reported to be completely atropine-resistant at low frequencies of nerve stimulation, partially at higher (Elmér 1975; Carpenter 1971). In the present study an atropine sensitive response was found already at the single shock level both in control and operated rats. To explain the partial atropine-resistant response it has been suggested that at low frequencies of nerve stimulation acetylcholine released acts predominantly on receptors within the neuromuscular junction not accessible to atropine while at high frequencies of stimulation large amounts of acetylcholine also diffuse to receptors which are sensitive to atropine located far away from the junction (Chesher 1970). Another explanation to the atropine resistance is the release of a transmitter other than acetylcholine (the presence of putative nerves releasing ATP has been discussed (Burnstock 1977)).

The atropine-resistant responses of the operated bladders were somewhat larger than those of the controls in the 1 week and 2-month series. Sensitization may perhaps be the explanation at the earliest time of observation and at the latest time of observation formation of new neuromuscular junctions less sensitive to atropine.

In the operated bladders belonging to the series of 1 and 2 months an α -adrenoceptor mediated inhibitory effect on the atropine resistant response was found at some frequencies. An α -adrenoceptor mediated inhibition of the transmission in the parasympathetic ganglia of the cat urinary bladder has been shown by de Groat & Saum (1971). However in the rat urinary bladder ganglia are only occasionally found (Carpenter & Rubin 1967; Chesher 1967; El-Badawi & Schenk 1966; Aim &

er 1975). It may be hypothesized that the outgrowth of adrenergic nerves to the partially denervated bladder has led to a morphological arrangement favouring an inhibitory influence on the afferent fibres at the periphery.

This work was supported by grants from the Medical Research Council to J. E.

REFERENCES

- M. P. & ELMÉR, M. 1975. Adrenergic and cholinergic innervation of the rat urinary bladder. *Acta Physiol Scand* 94, 36-43.
- M. P. & ELMÉR, M. 1979. Adrenergic reinnervation of the deservated rat urinary bladder. *Experientia (Basel)* 35, 1347-1348.
- NNS, H., EKSTRÖM, J. & MANN, S. P. 1978. Rapid restoration of choline acetyltransferase activity in the partially deservated urinary bladder. *Acta Physiol Scand* 102, 47-48A.
- NNS, H., EKSTRÖM, J. & MANN, S. P. 1979. Recovery of choline acetyltransferase activity in the urinary bladder deprived of half its innervation. *Acta Physiol Scand* 109, 83-88.
- OWN, M. C. & DRONTON, R. 1978. Sprouting and reorganization of neuromuscular synapses in partially deservated mammalian muscles. *J. Physiol. (Lond.)* 278, 325-348.
- RNSTOCK, G. 1972. Parasympathetic nerves. *Pharmacol. Rev.* 24, 509-581.
- ARPENTER, F. G. 1977. Allosteric resistance and muscarinic receptors in the rat urinary bladder. *Brit. J. Pharmacol.* 59, 43-49.
- ARPENTER, F. G. & RUBIN, R. M. 1967. The motor innervation of the rat urinary bladder. *J. Physiol. (Lond.)* 192, 609-617.
- ESHER, G. B. 1976. Acetylcholine in extracts and perfusates and endogenous acetylcholine in the urinary bladder. *Agents and Actions* 1, 123-133.
- GROAT, W. C. & SAUM, W. R. 1971. Adrenergic inhibition in mammalian parasympathetic ganglia. *Nature New Biology* 231, 188-189.
- EDDS, M. V. 1953. Collateral nerve regeneration. *Quart. Rev. Biol.* 28, 260-276.
- EKSTRÖM, J. 1975. Choline acetyltransferase activity in the rat's heart and urinary bladder after chemical sympathectomy. *Acta Pharmacol. (Kbh.)* 36, 234-238.
- EKSTRÖM, J. 1978. Acetylcholine synthesis and its dependence on nervous activity. *Experientia (Basel)* 34, 1247-1253.
- EKSTRÖM, J. 1979. Supersensitivity of the rat urinary bladder following chemical sympathectomy. *Acta Pharmacol. (Kbh.)* 41, 377-384.
- EKSTRÖM, J. & ELMÉR, M. 1977. Choline acetyltransferase activity in the deservated urinary bladder of the rat. *Acta Physiol Scand* 101, 98-100.
- EKSTRÖM, J., ELMÉR, M. & BANNIS, H. 1978. Transient supersensitivity in the partially deservated urinary bladder of the rat. *Acta Pharmacol. (Kbh.)* 43, 318-322.
- EL-BADAWI, A. & SCHENK, E. A. 1966. Dual innervation of the mammalian urinary bladder. A histochemical study of the distribution of cholinergic and adrenergic nerves. *Amer. J. Anat.* 119, 405-423.
- ELMÉR, M. 1974. Inhibitory β -adrenoceptors in the urinary bladder of the rat. *Life Sci* 15, 773-780.
- ELMÉR, M. 1975a. Stimulation of adrenergic nerve fibres to the urinary bladder of the rat. *Acta Physiol Scand* 94, 517-521.
- ELMÉR, M. 1975b. Stimulation of autonomic nerves to the urinary bladder of the rat. *Experientia (Basel)* 31, 814-815.
- ELMÉR, M. 1978. Cholinergic mechanisms in the rat detrusor muscle. *Acta Pharmacol. (Kbh.)* 43 (Suppl. 63), 63-68.
- LANGLEY, J. N. & ANDERSON, H. K. 1995. The innervation of the pelvic and adjoining viscera. *J. Physiol. (Lond.)* 19, 71-84.
- LANGWORTHY, O. R. 1965. Innervation of the pelvic organs of the rat. *Investigative Urology* 2, 491-511.
- MURRAY, J. G. & THOMPSON, J. W. 1957. The occurrence and function of collateral sprouting in the sympathetic nervous system of the cat. *J. Physiol. (Lond.)* 135, 133-162.
- PURINGTON, P. T., FLETCHER, T. F. & BRADLEY, W. E. 1973. Gross and light microscopic features of the pelvic plexus in the rat. *Anat. Rec.* 175, 697-706.

The immediate phenomenon is the motor response which is triggered by stimulation of the intact pelvic nerves and is a reflexive motoric but voluntary action. It is thus triggered by sensory stimulation of the afferent nerves. A virtually immediate relaxation of the bladder is observed after the reflexive motoric response (Elmér 1970). The best results are obtained in the rat (Carpenter & Rubin 1967; Elmér 1970) using the transverse ligament of the bladder, that the motor response is in the cranial half of the bladder. A motor response was also seen in larger than that observed in the cranial half of the two nerves. The motor response at the same time as the motor response of the cranial nerves while in the motor nerves the pelvic bladder relaxation is motor response. The action of the afferent nerves, the motor response before the motor response. This may suggest that the sensory nerves to complete their post-reception while during the following period of time changes occur in the nerve cells. To support this evidence, the efficiency of the transmission of nerve impulses.

The present experiments on the rat urinary bladder (Ekström & Vinn 1970; Ekström, Elmér & Bross 1971) show that in the rat the following evidence, the bladder gain in weight. The gain is lost later in the study on significant difference in the weight between normal and operated bladders was found at the last observation time. The bladder weight increase after partial denervation and that after complete denervation (Ekström & Elmér 1971) is probably due to a similar cause, i.e. distension of the bladder due to difficulties in bladder-emptying. Thus the discrepancy of the slight gain in weight in the series of 1 month may be taken as a further sign of a successful reinnervation.

Unfortunately bladders partially denervated by removal of the pelvic ganglion have not been investigated as to changes in the distribution of acetylcholinesterase-positive nerves. However in such a denervated bladder of the rat an outgrowth of adrenergic nerves from the intact half of the bladder as judged by fluorescent technique has been found to have occurred when examined 6-9 weeks post-operatively (Alm & Elmér 1979). In contrast to the cholinergic the adrenergic nerves do not distribute bilaterally (Carpenter & Rubin 1967; Elmér 1975b). The bilateral distribution of the cholinergic nerves

is through a fine fibre condition. Several studies on sympathetic postganglionic (A. Thompson 1970) and skeletal muscle (Bross & Fromm 1971) organs such studies the response is favoured a sympathetic and motor nerves interplay in motor. In partially denervated skeletal muscle the motor response was observed. Inguinalis 1-2 days postoperatively it is similar abundance during the third week connected with the motor nerves the columnar found in motor and early in the second week after the re-innervation of the motor nerves. A response in function was found in the motor.

The motor response of the rat urinary bladder have been reported to be completely atropine resistant at low frequencies of nerve stimulation (Elmér 1970; Carpenter 1971). The motor response at atropine-resistant was found at the single shock level with an atropine-resistant rat. To explain the atropine-resistant response it has been suggested that at low frequencies of nerve stimulation change in the motor response is predominantly on the motor nerves the motor response not atropine resistant while a high frequencies of single large amounts of atropine also diffuse to the motor nerves the motor response located for atropine from the motor (Chesher 1970). Another explanation to the atropine-resistant is the release of a transmitter other than acetylcholine the release of noradrenaline releasing ATP has been suggested (Burnstock 1972).

The atropine-resistant responses of the operated bladders were somewhat larger than those of the normal in the 1 week and 1-month series. This may perhaps be the explanation to the earliest time of observation and at the latest time of observation formation of new neurogenic functions less sensitive to atropine.

In the operated bladders belonging to the series 1 and 1 month an α -adrenoceptor mediated inhibitory effect on the atropine-resistant response was found at some frequencies. An α -adrenoceptor mediated inhibition of the transmission in parasympathetic ganglia of the rat urinary bladder has been shown by de Groat & Saum (1971). However in the rat urinary bladder ganglia are occasionally found (Carpenter & Rubin 1967; El-Badawi & Schenk 1966; A.

er 1975). It may be hypothesized that the out-
th of adrenergic nerves in the partially denerv-
d bladder has led to a morphological as-
ement (favouring an inhibitory influence on the
fibre in the periphery
ork as supported by grants from the Medical
ity in Lund to J. E.

REFERENCES

- ELMER, M. 1971 Adrenergic and cholinergic innervation of the rat urinary bladder. *Acta Physiol Scand* 94, 36-45.
- ELMER, M. 1979 Adrenergic reinnervation of the denervated rat urinary bladder. *Experientia (Basel)* 35, 1367-1368.
- NG, H., EKSTRÖM, J. & MANN, S. P. 1978. Rapid restoration of choline acetyltransferase activity in the partially denervated urinary bladder. *Acta Physiol Scand* 102, 47-48A.
- NG, H., EKSTRÖM, J. & MANN, S. P. 1979 Recovery of choline acetyltransferase activity in rat urinary bladder deprived of half its innervation. *Acta Physiol Scand* 109, 85-88.
- OWN, M. C. & BRINTON, R. 1978. Sprouting and regression of neuromuscular synapses in partially denervated mammalian muscles. *J Physiol (Lond.)* 278, 325-348.
- STENSTOCK, G. 1972. Parasympathetic nerves. *Pharmacol Rev.* 4, 505-511.
- WINTER, F. G. 1977. Atropine resistance and muscarinic receptors in the rat urinary bladder. *Brit J Pharmacol* 59, 43-49.
- WINTER, F. G. & RUBIN, R. M. 1967. The motor innervation of the rat urinary bladder. *J Physiol (Lond.)* 191, 609-617.
- WISHER, G. B. 1970. Acetylcholine in extracts and perfused and endogenous acetylcholine in the urinary bladder. *Agents and Actions* 1, 123-132.
- GROAT, W. C. & SAUL, W. R. 1971. Adrenergic inhibition in mammalian parasympathetic ganglia. *Nature New Biology* 231, 188-189.
- EDDS, M. V. 1953. Collateral nerve regeneration. *Quant Rev Biol* 28, 260-276.
- EKSTRÖM, J. 1975. Choline acetyltransferase activity in the rat heart and urinary bladder after chemical sympathectomy. *Acta Pharmacol (Kbh.)* 36, 284-288.
- EKSTRÖM, J. 1978. Acetylcholine synthesis and its dependence on nervous activity. *Experientia (Basel)* 34, 147-153.
- EKSTRÖM, J. 1979. Sensitivity of the rat urinary bladder following chemical sympathectomy. *Acta Pharmacol (Kbh.)* 44, 377-384.
- EKSTRÖM, J. & ELMER, M. 1977. Choline acetyltransferase activity in the denervated urinary bladder of the rat. *Acta Physiol Scand* 101, 48-62.
- EKSTRÖM, J., ELMER, M. & BANYS, H. 1978. Transient supersensitivity of the partially denervated urinary bladder of the rat. *Acta Pharmacol (Kbh.)* 43, 318-322.
- EL-BADAWI, A. & SCHENK, E. A. 1966. Dual innervation of the mammalian urinary bladder. A histochemical study of the distribution of cholinergic and adrenergic nerves. *Amer J Anat* 119, 405-428.
- ELMER, M. 1974. Inhibitory β -adrenoceptors in the urinary bladder of the rat. *Life Sci* 15, 273-280.
- ELMER, M. 1975 a. Stimulation of adrenergic nerve fibres to the urinary bladder of the rat. *Acta Physiol Scand* 94, 517-521.
- ELMER, M. 1975 b. Stimulation of autonomic nerves to the urinary bladder of the rat. *Experientia (Basel)* 31, 814-815.
- ELMER, M. 1978. Cholinergic mechanism in the rat detrusor muscle. *Acta Pharmacol (Kbh.)* 43 (II), 63-68.
- LANOLEY, J. N. & ANDERSON, H. K. 1895. The innervation of the pelvic and adjoining viscera. *J Physiol (Lond.)* 19, 71-84.
- LANGWORTHY, O. R. 1965. Innervation of the pelvic organs of the rat. *Investigative Urology* 2, 491-511.
- MURRAY, J. G. & THOMPSON, J. W. 1957. The occurrence and function of collateral sprouting in the sympathetic nervous system of the cat. *J Physiol (Lond.)* 135, 133-160.
- PURINGTON, P. T., FLETCHER, T. M. & BRADLEY, W. E. 1973. Gross and light microscopic features of the pelvic plexus in the rat. *Anat Rec* 175, 697-706.

Neuronal VIP in salivary glands Distribution and release

LEIF UDDMAN, JAN FAHRENKRUG, LARS MALM, JAN ALUMETS
LEIF HÅKANSSON AND FRANK SUNDLER

Department of Otorhinolaryngology, University Hospital, Malmö; Departments of Histology and Pharmacology,
University of Lund, Lund, Sweden; and Departments of Clinical Chemistry,
Glostrup and Bispebjerg Hospitals, Copenhagen, Denmark

UDDMAN L., FAHRENKRUG J., MALM L., ALUMETS J., HÅKANSSON L. &
SUNDLER, F. Neuronal VIP in salivary glands. Distribution and release. *Acta Physiol
Scand* 1980; 110: 31-38. Received 13 Nov 1979. ISSN 0001-6772. Department of Otorhinolaryngology, University Hospital, Malmö; the Departments of Histology and Pharmacology, University of Lund, Sweden; and the Departments of Clinical Chemistry, Glostrup and Bispebjerg Hospitals, Copenhagen, Denmark.

Nerves containing vasoactive intestinal peptide (VIP) were observed in salivary glands of rat, cat and guinea pig. VIP nerves were numerous in the cat, while they are moderate in number in rat and guinea pig. The measured concentrations of immunoreactive VIP are in agreement with the immunohistochemical findings. Electrical stimulation of the trigeminal chorda (lingual nerve) which stimulates salivary secretion and local blood flow, resulted in marked elevation of VIP in the venous effluent from the submandibular gland. VIP was not measurable in saliva. Gel permeation chromatography of eluate from cat submandibular gland and from guinea pig plasma collected before and during nervous stimulation revealed one immunoreactive peak with an elution position identical to that of highly purified porcine VIP. The finding of neuronal VIP in salivary glands, its release upon nerve stimulation and its known effect on local blood flow support the view that VIP is a neurotransmitter in the salivary gland.

Key words: Neuropeptides, vasoactive intestinal peptide (VIP), salivary glands, immunohistochemistry, radioimmunoassay, nerve stimulation.

Vasoactive intestinal polypeptide (VIP) is a neuropeptide with a wide range of biological effects. Among them are vasodilation (Said & Mutt 1970; Larsson et al. 1976a; Kachelhoffer et al. 1976), relaxation of smooth muscle (Piper et al. 1970; Said et al. 1974) and stimulation of secretion in several exocrine glands (Barberet & Olszyna 1971; Said & Mutt 1977; Mahiklof & Said 1973; Konturek et al. 1976; Domschke et al. 1977; Lundberg-Jensen et al. 1978). VIP-containing neurones are numerous in the central nervous system (Larsson et al. 1976b; Fuxe et al. 1977; Loren et al. 1979) and in several peripheral organs, such as the gastrointestinal tract (Bryant et al. 1976; Larsson et al. 1976b; Sundler et al. 1976; Uddman et al. 1978), genito-urinary tract (Alm et al. 1977; Larsson et al. 1977, b) and upper respiratory tract (Uddman et al. 1978b, 1979). The neuroendocrine utilization of VIP suggests a role for VIP in neurotransmission and evidence is accumulating

that VIP is a transmitter (cf. Fahrenkrug 1979). In the present investigation we have examined the occurrence and distribution of VIP-containing nerves in salivary glands using immunohistochemical techniques and quantified the content by radioimmunoassay. Further, we have demonstrated a release of VIP into local venous effluent upon stimulation of the chorda lingual nerve.

MATERIAL AND METHODS

Tissue material

Tissue specimens were taken from the parotid, submaxillary, extramorbilary lacrimal and sublingual glands of 8 rats and from the parotid, submandibular, sublingual and zygomatic glands of 14 cats. The rats were killed by decapitation under diethyl ether anaesthesia and the cats by asphyxiation following an overdose of pentobarbital sodium. In addition, tissue material classified as normal by visual inspection was collected from 10 patients undergoing parotid gland surgery due to benign tumours. Salivary gland tissue was also obtained from 10 patients

neuronal VIP in salivary glands Distribution and release

J. UDDMAN, JAN FAHRENKRUG, LARS MALM, JAN ALUMETS,
F. HÅKANSON AND FRANK SUNDLER

Department of Otolaryngology, University Hospital, Malmö; Departments of Histology and Pharmacology,
University of Lund, Lund, Sweden; and Departments of Clinical Chemistry,
Glostrup and Bispebjerg Hospitals, Copenhagen, Denmark

UDDMAN J., FAHRENKRUG J., MALM L., ALUMETS J., HÅKANSON F. &
SUNDLER, F. Neuronal VIP in salivary glands. Distribution and release. *Acta Physiol
Scand* 1980, 110: 31-38. Received 13 Nov. 1979. ISSN 0001-6772. Department of Oto-
laryngology, University Hospital, Malmö; the Departments of Histology and Phar-
macology, University of Lund, Sweden; and the Department of Clinical Chemistry, Glo-
strup and Bispebjerg Hospitals, Copenhagen, Denmark.

Nerves containing vasoactive intestinal peptide (VIP) are observed in salivary glands of
rat, cat and man. VIP nerves are numerous in the cat while they were moderate in number
in rat and man. The measured concentrations of immunoreassayable VIP were in agreement
with the immunohistochemical findings. Electrical stimulation of the feline chorda lingual
nerve which stimulates salivary secretion and local blood flow resulted in marked
elevation of VIP in the venous effluent from the submandibular gland. VIP was not
measurable in saliva. Gel permeation chromatography of extract from cat submandibular
gland and from venous plasma collected before and during nervous stimulation revealed
one immunoreactive peak with an elution position identical to that of highly purified
porcine VIP. The finding of neuronal VIP in salivary gland, its release upon nerve
stimulation and its known effect on local blood flow support the view that VIP is
neurotransmitter in the salivary glands.

Key words: Neuropeptides, vasoactive intestinal peptide (VIP), salivary glands, im-
munohistochemistry, radioimmunoassay, nerve stimulation.

Vasoactive intestinal polypeptide (VIP) is a
neuropeptide with a wide range of biological ef-
fects. Among them are vasodilation (Said & Mutt
1970; Larsson et al. 1976a; Kachelhoffer et al.
1976), relaxation of smooth muscle (Piper et al.
1970; Said et al. 1974) and stimulation of secretion
from several exocrine glands (Barberat &
Ossola 1971; Said & Mutt 1972; Mahlkouf &
Kodurek et al. 1976; Doroschke et al.
1977; Lamläsa-Jensen et al. 1978). VIP-containing
nerves are numerous in the central nervous sys-
tem (Larsson et al. 1976b; Fuxe et al. 1977; Lorén
et al. 1979) and in several peripheral organs such as
the gastrointestinal tract (Bryant et al. 1976;
Larsson et al. 1976a; Sundler et al. 1976; Uddman
et al. 1978a), genito-urinary tract (Ahn et al. 1977;
Larsson et al. 1977a, b) and upper respiratory
tract (Uddman et al. 1978b, 1979). The neuronal
calculation of VIP suggests a role for VIP in
neurotransmission and evidence is accumulating

that VIP is a transmitter (cf. Fahrenkrug 1979). In
the present investigation we have examined the oc-
currence and distribution of VIP-containing nerves
in salivary glands using immunohistochemical
techniques and quantified the content by radioim-
munoassay. Further, we have demonstrated a re-
lease of VIP into local venous effluent upon
stimulation of the chorda lingual nerve.

MATERIAL AND METHODS

Tissue material

Tissue specimens were taken from the parotid, subman-
dibular, extraparietal lacrimal and sublingual glands of 8 rats
and from the parotid, submandibular, sublingual and
zygomatic glands of 14 cats. The rats were killed by de-
capitation under diethyl ether anaesthesia and the cats by
exsanguination following an overdose of pentobarbital
sodium. In addition, tissue material classified as normal
by visual inspection was collected from 10 patients under-
going parotid gland surgery due to benign tumours.
Salivary gland tissue was also obtained from 10 patients

undergoing submandibular gland surgery due to ductal obstruction

Immunohistochemical procedures

All specimens were frozen to the temperature of liquid nitrogen in a propane-propylene mixture and freeze-dried. They were then fixed in diethylpyrocarbonate vapour at 55°C for 3 h (Pearse et al. 1974) and embedded in paraffin in vacuo. Deparaffinized sections were processed for the immunohistochemical demonstration of VIP using the indirect immunofluorescence procedure (Coons et al. 1955) or the peroxidase-antiperoxidase (PAP) technique (Sternberger 1974). The VIP antiserum (code no. 98P and 3603) were raised against highly purified porcine VIP and have been characterized in detail elsewhere (Säid & Faloona 1973; Fahrenkrug & Schaffalitzky de Muckadeil 1977, 1978). In cat and man VIP nerves were demonstrated using 3603 while 98P was used in the rat. Antiserum 3603 was used in dilution 1:80 (immunofluorescence) or 1:5280 (PAP staining). Antiserum 98P (a kind gift from Dr S. J. Säid Dallas, Texas, USA) was used in dilution 1:320 (PAP staining). Fluorescein isothiocyanate labelled sheep anti rabbit IgG serum was used in dilution 1:20 (SBL, Stockholm, Sweden). PAP complex (Cappel Labs, Downingtown Pa, USA) was used in dilution 1:320. Sections incubated with antiserum inactivated by the addition of excess antigen (30 nmol of highly purified porcine VIP per ml diluted antiserum) served as controls.

Nervous release of VIP

6 adult cats were anesthetized with α -chloralose (80 mg/kg i.v.) after induction with diethylether. Spontaneous breathing was ensured by inserting a tracheal cannula. Rectal temperature was kept at 37–38°C by means of an electrically heated operation table. A glass cannula was inserted into the submandibular duct distal to the point at which it is crossed by the lingual nerve. Chorda lingual nerve was exposed as far centrally as possible and the peripheral end was mounted on bipolar electrodes. Stimuli were delivered by a Grass model S44 stimulator. The nerve was stimulated with square wave pulses with an intensity which was at least twice the threshold value (6–8 V, ms). Stimulations in the following order of frequencies: 20, 10, 5, 2.5 and again 20 Hz, were used with resting period of 15 min between each stimulation. The peripheral end of the cut cervical sympathetic nerve was also stimulated (10 Hz, 4–6 V, ms) after the first chorda lingual nerve stimulation. A expected stimulation of the chorda lingual nerve increased coronary blood flow and salivary flow (Hilton & Lewis 1954).

In order to demonstrate a release of VIP into the local venous effluent during stimulation all tributaries to the external jugular vein except one or two veins from the submandibular gland were ligated. Heparin (1000 I.U./kg i.v.) was given and the external jugular vein was cannulated with a polyethylene catheter. The animal was then left undisturbed for 30–60 min. During each stimulation period 5 ml of blood was collected in ice-chilled tubes containing 2 500 k.I.U. of the protease inhibitor aprotinin (Trasyol, Bayer AG, Leverkusen, FRG). The first 3–6 drops of each stimulation were discarded to avoid con-

tamination from the previous unstimulated period between the stimulation periods the venous effluent returned to the circulation via the femoral vein. The blood were centrifuged immediately after 5 ml of blood obtained. Plasma was pipetted off and stored at -20°C until assayed for VIP. Saliva was collected from the stimulated submandibular gland with the same procedure for plasma.

Radioimmunoassay

The concentration of VIP in salivary gland tissue was measured radioimmunochemically (Fahrenkrug & Schaffalitzky de Muckadeil 1977, 1978). The specimens were immediately frozen on dry ice and weighed. The frozen tissue was stored at -20°C. Extraction of VIP by homogenization at 0°C in a homogenizer containing 5 vol of acidified ethanol (ethanol containing 0.74% hydrochloric acid) followed by centrifugation (1500 g) at 4°C for 15 min the supernatant was decanted and the residue re-suspended and homogenized with additional 5 vol of acidified ethanol. After centrifugation the combined supernatant fractions were evaporated to dryness in vacuo. The samples were reconstituted and diluted in 0.04 M sodium phosphate buffer pH 7.4 containing 58 μ mol human serum albumin (Behringwerke, Marburg am Lahn, FRG) and 0.1 M sodium chloride and assayed in 5 different dilutions. Plasma samples were extracted with 1 vol of acidified ethanol as previously described (Fahrenkrug & Schaffalitzky de Muckadeil 1977).

Gel permeation chromatography

In order to compare the chromatographic behaviour of the immunoreactive VIP with highly purified porcine VIP (kind gift from Prof. V. Müll, Karolinska Institute, Stockholm, Sweden) gel permeation chromatography was performed on extracts of the feline submandibular gland and of plasma from the venous effluent of cat submandibular gland before and during nervous stimulation. Samples were extracted as described above. 500 μ l mixed with trace amounts of α -albumin and α -actin were applied to Sephadex G-50 superfine columns (11 \times 600 mm). Elution was performed at 4°C with 0.05 M ammonium acetate pH 6.5 containing 7.5 μ mol human serum albumin (Behringwerke, Marburg am Lahn, FRG) per liter at a flow rate of 1 ml per h. Fractions of 5 ml were collected and the concentration of immunoreactive VIP in each fraction was measured.

Statistics

Statistical analysis was performed using Student's *t*-test for paired observation.

RESULTS

Immunohistochemistry

VIP immunoreactive nerve were detected in the salivary glands of all species examined. The distribution of VIP nerves within the gland was markedly patchy with some areas totally devoid of immunoreactive nerves. This was particularly conspicuous in salivary glands of man. The VIP nerves

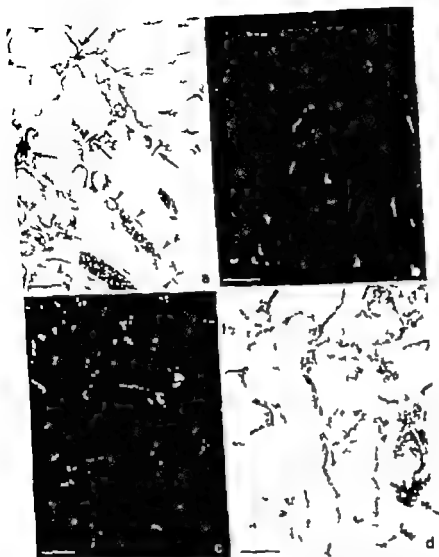


Fig. 1. Distribution of VIP nerves in salivary glands. (a) Rat parotid gland. Few scattered fine varicose VIP nerves in the wall of blood vessels (arrow heads) and close to acini (arrows). (b-d) Cat sublingual (b), submandibular (c) and parotid (d) gland. Rich supply of VIP nerves around acini, blood vessels (—) and around salivary duct (d) (—) and (d) PAP-staining. (b) and (c) immunofluorescence. Marker 50 µm.

were seen to surround glandular acini and to run close to small blood vessels. A notable finding was the accumulation of VIP nerves around salivary ducts where they formed dense plexa beneath the epithelium. Occasionally immunoreactive nerves were observed in the connective tissue stroma within the glandular parenchyma. In the rat the number of immunoreactive nerves was moderate in all salivary glands (Fig. 1). In the cat VIP nerves were numerous in the sublingual (Fig. 1b) and sub-

mandibular glands (Fig. 1c) and the parotid glands (Fig. 1d) the VIP nerves had a patchy distribution and were somewhat less numerous. They were few in the zygomatic glands. In man the frequency of VIP nerves was moderate in both the parotid and submandibular glands.

The main excretory ducts of feline parotid, submandibular and sublingual glands received a particularly rich supply of VIP nerves (Fig. 2a). In the human region of the submandibular gland, chus-



Fig 2 Cat submandibular gland (a) Main excretory duct surrounded by numerous VIP nerves located beneath epithelium (b) Hilar region. A small ganglion containing numerous nerve cell bodies and nerve fibres displaying a degree of VIP immunoreactivity (a) Immunofluorescence ($\times 150$) (b) PAP staining. Marker 40 μm

ters of VIP immunoreactive nerve cell bodies were encountered (Fig. 2b)

VIP concentration in salivary gland tissue

The concentration of immunoreactive VIP in the various salivary glands of rat, cat and man are given in Table 1. On the whole the values agreed well with the histochemical picture. The highest concentrations were found in the sublingual and submandibular gland of cats while the parotid and zygomatic glands contained smaller amounts of VIP. In man immunoreactive VIP was found in larger amounts in the submandibular gland than in the parotid gland while in rats the concentration was moderate and about the same in the different salivary glands.

Nervous release of VIP

Electrical stimulations of the chorda lingual nerve raised the plasma concentration of immunoreactive VIP in the venous effluent (Fig. 3). Plasma VIP was unaffected by stimulation of the cervical sym-

pathetic nerve. Initial experiments demonstrated VIP-like immunoreactivity in saliva. This was however later found to be due to protein interference in the radioimmunoassay. Consequently VIP does not seem to be released into saliva.

Gel permeation chromatography

Gel-permeation chromatography of extracts from cat submandibular gland (Fig. 4a) and of venous plasma (Fig. 4b) collected before and during chorda lingual nerve stimulation invariably revealed a single immunoreactive peak with an elution position identical to that of highly purified porcine VIP.

DISCUSSION

The occurrence of VIP-containing nerves in salivary glands has been noted in previous studies (Bryant et al 1976; Wharton et al 1979). The present investigation confirms and extends these morphological observations. In contrast to these

Table 1 VIP concentration (pmol/g) in various salivary glands

Mean \pm S.E. (n). n is the number of glands analysed. n.d. = not determined. — = non-existent

Species	Parotid gland	Submandibular gland	Sublingual gland	Zygomatic gland	Extraorbital lacrimal gland
Rat	11 ± 1 (3)	38 ± 7 (3)	n.d.	—	70 ± 7 (3)
Cat	11 ± 3 (15)	223 ± 43 (18)	153 ± 76 (1)	39 ± 6 (1)	—
Man	8 ± 3 (8)	44 ± 3 (8)	n.d.	—	—

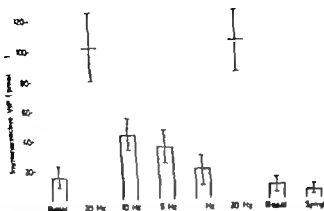


Fig. 3 Plasma VIP in venous effluent from the submandibular gland of 6 cats during stimulation of the chorda lingual nerves or the cervical sympathetic nerve and during unstimulated periods (basal). Vertical bars gives S.E.M. The concentration was increased at stimulation with 20 Hz compared to basal values ($P < 0.01$) and also to values obtained with stimulation at lower frequencies ($P < 0.05$). Stimulation was performed in the sequence indicated in the figure.

as we find great species differences in that VIP nerves were frequent in the parotid submandibular and sublingual glands of the cat, less frequent in the parotid and submandibular glands of man and scarce in the salivary glands of rat. Species differences in distribution have also been noted for adrenergic and cholinergic nerves (Soell 1958, Norberg & Olson 1965, Garrett 1966, 1967). On the whole, the frequency and distribution of VIP nerves

agreed fairly well with the concentrations of VIP measured by radioimmunoassay in extract of the salivary glands.

The VIP nerves were distributed around glandular acini and small blood vessels. A particularly rich VIP nerve supply was noted around the intraparenchymal salivary ducts and the main excretory ducts. The presence of VIP immunoreactive nerve cell bodies in ganglia in the hilar region of the sub-

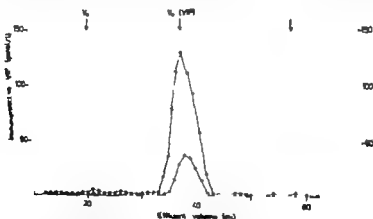


Fig. 4 Electrochromatogram of VIP immunoreactivity in plasma samples obtained from the excretory effluent of the cat submandibular gland before (open circles) and during stimulation (8V, 5 Hz, 2 ms) of the chorda lingual nerve (closed circles). Gel-permeation chromatography on Sephadex G-50 superfine column (II 600 mm) eluted at 4°C with 0.25 M ammonium acetate, pH 6.5 containing 72.5 μ mol bovine serum albumin at a flow rate of 1.2 ml/h. The column was calibrated with 125 I-albumin, 22 NaCl and highly purified porcine VIP. V_0 , void volume; V_m , elution position of highly purified porcine VIP; V , total mobile phase.

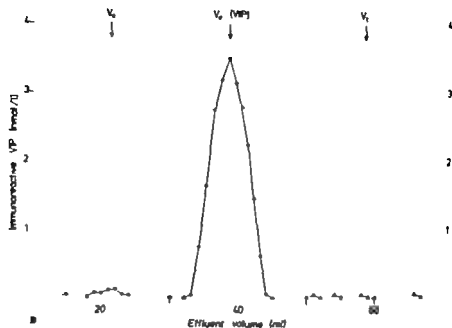


Fig. 4b. Elution diagram of VIP immunoreactivity from the feline submandibular gland extracted with acidified ethanol. (For further details see Methods.)

mandibular gland suggests that at least some of the VIP nerves in this gland may derive from local ganglia. Numerous VIP nerves have previously been noted around excretory ducts of other exocrine glands and in particular around sphincters (Alumets et al. 1979). It is conceivable that the VIP nerves around the excretory ducts of the salivary glands participate in the regulation of the ejection of saliva and its electrolyte composition (Dennis & Young 1978). Under basal conditions VIP has no stimulatory effect on salivary secretion (Hedemark et al. unpublished observations).

Electrophysiological and pharmacological studies have indicated that sympathetic and parasympathetic nerves take part in regulating local blood flow and secretion in salivary glands (cf. Burgen & Emmelin 1961). An atropine resistant vasodilation has been observed upon stimulation of the chorda lingual nerve. The nervous mediator of this action is unknown (Hilton & Lewis 1955; Bhoola et al. 1965; Gautvik 1967; Skinner & Webster 1968) but VIP is a likely candidate based on the following lines of evidence: (1) the effect of VIP on salivary blood flow (Shimizu & Taira 1979) mimics chorda lingual nerve stimulation; (2) VIP occurs in nerves around the blood vessels of the salivary glands; (3) electrical stimulation of fibres in the chorda lingual nerve causes release of VIP in the venous effluent.

This study was supported by grants from the Swedish Medical Research Council (04X-4499) the Danish Medical

Research Council (J. No. B1093) Pihl von S. Foundation and P. Carl Petersen's Foundation.

REFERENCES

- ALM, P., ALUMETS, J., HÅKANSON, R. & SUNDLER, F. 1977. Peptidergic (VIP) nerves in the genito-urinary tract. *Neuroscience*, **2**, 731-754.
- ALM, P., ALUMETS, J., HÅKANSON, R., ÖRSKOV, C. H., SJÖBERG, N.-O., SUNDLER, F. & WALLIN, B. 1979. Origin and distribution of VIP nerves in the genito-urinary tract. *Cell Tissue Res.* **205**, 337-347.
- ALUMETS, J., FAHRENKRUG, J., HÅKANSON, R., SCHIAFFALITZKY, DE MUCKADELL, B. & SUNDLER, F. & UDDMAN, R. 1979. A novel nerve supply is characteristic of sphincters. *Nature (Lond.)* **280**, 155-156.
- BARBEZAT, G. O. & GROSSMAN, M. I. 1971. Intranasal secretion: stimulation by peptides. *Science* **171**, 444-445.
- BHoola, K. D., MORLEY, J., SCHACHTER, M. J. & SMAJE, L. H. 1965. Vasodilation in the sublingual gland of the cat. *J. Physiol.* **119**, 172-184.
- BRYANT, M. G., BLOOM, S. R., POLAK, J. M., ALBUQUERQUE, R. H., MODLIN, I. & PEARSE, A. G. E. 1976. Possible dual role for vasoactive intestinal peptide as a gastrointestinal hormone and neurotransmitter substance. *Lancet* **i**, 911-993.
- BURGEN, A. S. V. & EMMELIN, N. G. 1961. Physiology of the salivary gland. E. Arnold Ltd, London.
- COONS, A. H., LEDUC, E. H. & CONOLLY, J. V. 1955. Studies of antibody production. I. A method for the histochemical demonstration of specific antibody and its application to study of the hyperimmune rabbit. *J. Exp. Med.* **102**, 49-60.
- DENNISS, A. R. & YOUNG, J. A. 1978. Modification of salivary duct electrolyte transport in rat and rabbit.

- thyliarion, VIP, GIP and other enterohormones. *Magasin Arch* 376 73-80.
- MSCHKE, S., DOMSCHKE, W., RÖSCH, W., KONTUREK, S. J., SPRÜGEL, W., MITZNEGG, W., WUNSCH, E. & DEMLING, L. 1977. Vasointestinal peptide: A secretin-like partial agonist for pancreatic secretion in man. *Gastroenterology* 73 473-480.
- HRENKRUG, J. 1979. Vasointestinal polypeptide: measurement, distribution and putative neurotransmitter function. *Digestion* 19 149-169.
- HRENKRUG, J. & SCHAFFALITZKY DE MUCKADELL, O. B. 1977. Radioimmunoassay of vasoactive intestinal polypeptide (VIP) in plasma. *J Lab Clin Med* 89 1379-1388.
- HRENKRUG, J. & SCHAFFALITZKY DE MUCKADELL, O. B. 1978. Distribution of vasoactive intestinal polypeptide (VIP) in the porcine central nervous system. *J Neurochem* 31 1445-1451.
- HRENKRUG, J., HAGLUND, U., JODAL, M., LUNDGREN, O., OLBE, L. & SCHAFFALITZKY DE MUCKADELL, O. B. 1978. Nervous release of vasoactive intestinal polypeptide (VIP) in the gastrointestinal tract of cats. Possible physiological implications. *J Physiol* 284 291-305.
- JXE, K., HOKFELT, T., SAID, S. I. & MUTT, V. 1977. Vasoactive intestinal polypeptide and the serotonin system: Immunohistochemical evidence for localization in central and peripheral neurons, particularly intracortical neurons of the cerebral cortex. *Neuroscience Letters* 5 41-46.
- ARRETT, J. R. 1966. The innervation of salivary glands. Part I. Cholinesterase-positive nerves in normal glands of the cat. *J R Micro Soc* 85 135-148.
- ARRETT, J. R. 1967. The innervation of normal human submandibular and parotid salivary glands. *Arch Oral Biol* 12 117-136.
- AUTVIK, K. 1969. Parasympathetic neuro-effector transmission and functional vasodilatation in the submandibular salivary gland of cats. *Acta Physiol Scand* 79 204-215.
- HILTON, S. M. & LEWIS, G. P. 1975. The cause of the vasodilatation accompanying activity in the submandibular salivary gland. *J Physiol* 128 235-48.
- SACHSELHOFFER, J., ELOY, M. R., POUSSÉ, A., HOHNATTELL, D. & GRENIER, J. P. 1974. Motoric vasomotor effect of vasoactive intestinal polypeptide: study on perfused isolated canine jejunal loops. *Magasin Arch* 352 37-46.
- KONTUREK, S. J., FUCHER, A. & RADECKI, T. 1976. Comparison of vasoactive intestinal peptide and secretin in stimulation of pancreatic secretion. *J Physiol* 255 479-509.
- LARSSON, L. I., FAHRENKRUG, J. & SCHAFFALITZKY DE MUCKADELL, O. B. 1977. Occurrence of nerves containing vasoactive intestinal polypeptide immunoreactivity in the male genital tract. *Life Sci* 21 363-368.
- LARSSON, L. I., FAHRENKRUG, J. & SCHAFFALITZKY DE MUCKADELL, O. B. 1978. Vasoactive intestinal polypeptide occurs in nerves of the female genital urinary tract. *Science* 197 1374-1375.
- LARSSON, L. I., FAHRENKRUG, J., SCHAFFALITZKY DE MUCKADELL, O. B., SUNDLER, F., HÅKANSON, R. & REHFELD, J. F. 1976b. Localization of vasoactive intestinal polypeptide (VIP) to central and peripheral neurons. *Proc Natl Acad Sci USA* 73 3197-3200.
- LARSSON, L. I., EDVINSSON, L., FAHRENKRUG, J., HÅKANSON, R., ÖRTENGREN, C., SCHAFFALITZKY DE MUCKADELL, O. B. & SUNDLER, F. 1976a. Immunohistochemical localization of vasodilatory polypeptide (VIP) in cerebrovascular nerves. *Brain Res* 113 400-403.
- LINDKÆR-JENSEN, S., FAHRENKRUG, J., HOLST, J. J., VAGN, NIELSEN, D. & SCHAFFALITZKY DE MUCKADELL, O. B. 1978. Secretory effect of VIP on isolated perfused porcine pancreas. *Am J Physiol* 235 E387-E391.
- LÖREN, I., ERIKSSON, P. C., FAHRENKRUG, J., BJÖRKLUND, A., ALUMETS, J., HÅKANSON, R. & SUNDLER, F. 1979. Distribution of vasoactive intestinal polypeptide in the rat and mouse brain. *Neuroscience* 4 1953-1976.
- MAKHLOUF, O. M. & SAID, S. I. 1975. The effect of vasoactive intestinal peptide (VIP) on digestive and hormonal function. In: *Gastrointestinal hormones* (ed. J. C. Thompson) pp. 599-610. University of Texas Press, Austin.
- NORBERG, K. A. & OLSON, L. 1965. Adrenergic innervation of the salivary glands in the rat. *Z Zellforsch* 68 183-189.
- PEARSE, A. H. E., POLAK, J., ADAMS, C. & KENDALL, P. A. 1974. Dietary pyrocarbonate, vapour phase fluorine for immunofluorescence studies on polypeptide hormones. *Histochem J* 6 347-352.
- PIPER, P. J., SAID, S. I. & VANE, J. R. 1970. Effect on smooth muscle preparations of unidentified vasoactive peptides from intestine and lung. *Nature* 225 1144-1146.
- SAID, S. I. & FALOONA, R. 1973. Elevated plasma and tissue levels of vasoactive intestinal polypeptide in the watery-diarrhea syndrome due to pancreatic, bronchogenic and other tumors. *New England J Med* 289 155-160.
- SAID, S. I. & MUTT, V. 1970. Polypeptide with broad biological activity: isolation from small intestine. *Science* 169 1217-1218.
- SAID, S. I. & MUTT, V. 1972. Isolation from porcine-intestinal wall of vasoactive octacosapeptide related to secretin and to glucagon. *Eur J Biochem* 28 199-204.
- SAID, S. I., KITAMURA, S., YOSHIDA, T., PRESKITT, J. & HOLDEN, L. D. 1974. Humoral control of airways. *Ann NY Acad Sci* 221 103-114.
- SHIMIZU, T. & TAIRA, N. 1979. Assessment of the effects of vasoactive intestinal peptide (VIP) on blood flow through and innervation of the dog salivary gland in comparison with those of secretin, glucagon and acetylcholine. *Br J Pharmacol* 63 663-667.
- SKINNER, N. S. & WEBSTER, M. E. 1968. Kinetic, beta-adrenergic receptors and functional vasodilatation in the submandibular gland of the cat. *J Physiol* 195 505-519.

- SNELL, R. S. 1958. The histochemical appearances of cholinesterase in the parasympathetic nerves supplying the submandibular and sublingual salivary glands of the rat. *J Anat* 62: 534-543.
- STERNBERGER, L. A. 1974. *Immunocytochemistry*. Prentice-Hall Inc. Englewood Cliffs, NJ.
- SUNDLER, F., ALUMETS, J., HÅKANSON, R., FAHRENKRUG, J. & SCHAFFALITZKY DE MUCKADELL, O. B. 1978. Peptidergic (VIP) nerves in the pancreas. *Histochemistry* 55: 173-176.
- UDDMAN, R., ALUMETS, J., EDVINSSON, L., HÅKANSON, R. & SUNDLER, F. 1978a. Peptidergic (VIP) innervation of the esophagus. *Gastroenterology* 75: 5-8.
- UDDMAN, R., ALUMETS, J., EDVINSSON, L., HÅKANSON, R. & SUNDLER, F. 1978b. Presence and distribution of VIP nerves in the mucosa and tracheobronchial wall. *Acta Otolaryngol (Stockh)* 85: 443-448.
- UDDMAN, R., MALM, I. & SUNDLER, F. 1979. Origin of vasoactive intestinal polypeptide (VIP) nerves in the feline nasal mucosa. *Acta Otolaryngol (Stockh)* 89: 15-19.
- WHARTON, J., POLAK, J. M., BRYANT, M. G., NOORDEN, S., BLOOM, S. R. & PEARSE, A. G. 1979. Vasoactive intestinal polypeptide (VIP) immunoreactivity in salivary glands. *Life Sci* 25: 73-79.

Transcapillary exchange and distribution of carbon 11 labelled ethanol in the isolated perfused rat liver

KEINÄNEN J, KUIKKA S, J. HESELIUS E, M. SUOLINNA E, M. SOLIN B, LÄNGSTRÖM B and V. NÄNTÖ

Medical Cyclotron Project, University of Turku and Åbo Akademi, Turku, Finland
Department of Clinical Physiology, University Central Hospital, Kuopio, Finland
Department of Organic Chemistry, University of Uppsala, Sweden

KEINÄNEN M., KUIKKA J., HESELIUS, S. J., SUOLINNA E. M., SOLIN B., LÄNGSTRÖM B. & NÄNTÖ V. Transcapillary exchange and distribution of carbon-11 labelled ethanol in the isolated perfused rat liver. *Acta Physiol Scand* 1980, 110: 39-46. Received 15 November 1979. ISSN 0001-0709. Medical Cyclotron Project, University of Turku and Åbo Akademi, Turku, Department of Clinical Physiology, University Central Hospital, Kuopio, Finland and Department of Organic Chemistry, University of Uppsala, Sweden.

The distribution kinetics of ethanol in the isolated perfused rat liver at various flow rates and ethanol concentrations were studied by the indicator diffusion technique using ^{11}C labelled ethanol. A double tracer solution with ^{14}C -labelled red blood cells as the vascular reference and ^{11}C -ethanol as the test substance was injected into the catheter connected to the portal vein. Time-activity curves were monitored by NaI(Tl) scintillation detector above the outflow catheter. The following values were obtained: 0.95 ± 0.01 for the maximal extraction (E_{max}), 3.0 ± 0.1 flow ($r=0.96$) for the capillary permeability surface area product (PS_{cap}), $18.7 \pm 2.3\%$ for the vascular volume (V_v) and $56.2 \pm 9.0\%$ for the ethanol distribution volume (V_d). The volumes were calculated from the products of mean transit time (MTT) and flow (Q), and PS_{cap} was calculated from the equation $PS_{\text{cap}} = -f \ln(1 - E_{\text{max}})$. The results show that the ethanol distribution in the liver is flow limited since E_{max} was constant at various flow rates ($0.67-2.35$ ml/g min). The distribution volumes obtained agree well with the values for liver vascular volume and water distribution volume reported in the literature.

Key words: Ethanol distribution kinetics, liver blood flow, capillary permeability, diffusible indicators, cyclotron produced isotopes.

Ethanol is a fast diffusible non-electrolyte which penetrates cell membranes passively and is distributed in the body to the water distribution volume (e.g. Wallgren & Barry 1970). Absorption, distribution and elimination of ethanol in the body have been studied by monitoring its blood levels (e.g. Widmark 1932, Arnbomsen & Crone 1956, Moore 1963) and by tissue analysis techniques (e.g. Kulonen & Forsander 1959, Åkesson 1974, Enckson 1976, Scherrer, Eberne & Posternak 1963). Since ethanol is eliminated mainly by hepatic metabolism, the study of its metabolism and kinetics in normal and damaged liver is of importance. There are studies on ethanol consumption in the isolated perfused liver (e.g. Forsander 1960, Gordon 1966) but transcapillary exchange and distribution kinetics have not been investigated.

Carbon-11, a short lived cyclotron produced isotope ($T_{1/2} = 20.4$ min, gamma energy 0.511 MeV) has many advantages in biological and medical studies (Tilbury 1974, Wolf 1977): the isotope can be detected externally, the radiation dose is small and the experiment can be repeated after short intervals. Synthesis of ^{11}C -labelled ethanol is relatively simple but thus far its experimental use has been scarce. De Grazia et al. (1975) have given brief report on the whole body distribution of ^{11}C ethanol in cats. Raichle et al. (1976) have stated that ^{11}C -ethanol extraction during the first capillary passage was 97% in the brain of monkeys. Robinson et al. (1973) have studied ^{11}C -ethanol extraction in the brain and Poe et al. (1973) in the heart, but they do not introduce quantitative results.

We use ^{11}C -ethanol to investigate extraction and

- SNELL R S 1958 The histochemical appearances of cholinesterase in the parasympathetic nerves supplying the submandibular and sublingual salivary glands of the rat *J Anat* 62 534-543
- STERNBERGER L A 1974 Immunocytochemistry Prentice Hall Inc Englewood Cliffs, N J
- SUNDLER F ALUMETS J HÅKANSON R FAHRENKRUG J & SCHAFFALITZKY DE MUCKADELL O B 1978 Peptidergic (VIP) nerves in the pancreas *Histochemistry* 55 173-176
- UDDMAN R ALUMETS J EDVINSSON L HÅKANSON R & SUNDLER F 1978a Peptidergic (VIP) innervation of the esophagus *Gastroenterology* 75 5-8
- UDDMAN R ALUMETS J DENSEN HÅKANSON R & SUNDLER F 1978b Presence and distribution of VIP nerves in the mucosa and tracheobronchial wall *Acta Otol (Stockh)* 85 443-448
- UDDMAN R MALM L & SUNDLER F 1979 origin of vasoactive intestinal polypeptide nerves in the feline nasal mucosa. *Acta Otol (Stockh)* 89 157-156
- WHARTON J POLAK J M BRYANT M G NOORDEN S BLOOM S R & PEARSE A C 1979 Vasoactive intestinal polypeptide (VIP) immunoreactivity in salivary glands. *Life Sci* 25 73-280

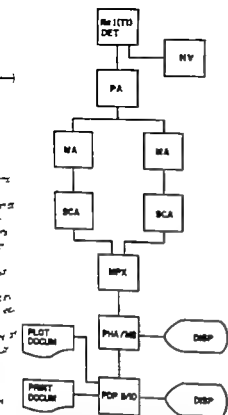


Fig. 3. Flow chart of the radioactivity counting device and computer hardware. Abbreviations: NaI DET = detector; HV = high voltage supply; PA = pre-amplifier; MA = multiplier amplifier; SCA = single channel analyzer; MPX = multiplexer; PHA/MS = pulse height analyzer in tracking mode; PLOT DECU = plotter; DISP = display; PDP 11/10 = computer.

sampler and two single channel analyzers using counting unit (Fig. 3). The whole photopile was used in window setting. A 40% channel pulse height analyzer window setting mode (Nuclear Data Inc.) was used for setting. A channel width of 0.4 was used. The whole counting time was 5 min. From the memory of the pulse height analyzer the data were transferred to PDP 11/10 computer (Digital Equipment Corp. Ltd.) and stored on magnetic disc. For the calculations corrections were made to the background counting rates, physical half-life of ^{51}Cr -H and overlapping of ^{51}Cr -activity to the ^{51}Cr -activity (9.2). The catheter delay was corrected according to the method of (1966). The peak counting rates were about 4000 counts/s for ^{51}Cr -red blood cells and 2000 counts/s for ^{51}Cr -ethanol.

The radioactivity-time curves for ^{51}Cr -red blood cells and ^{51}Cr -ethanol are normalized to unity area to give the transport functions $h_1(t)$ and $h_2(t)$ used in the calculations.

The digital data were analysed with a Basic program. The following parameters were calculated: the maximal ethanol extraction (E_{max}) and capillary permeability surface area product (PS_{cap}) (Crone 1963) mean transit times (MTT) and mean transit time distribution volumes ($V = {}^{51}\text{Cr}$ -RBC distribution volume $V = {}^{51}\text{Cr}$ -ethanol distribution volume) (Goresky 1963 1970 and Benavente 1970 1974). The following equations were used:

(1) Instantaneous extraction,

$$(E(t)) = \frac{h_1(t) - h_2(t)}{h_2(t)}$$

where $h_1(t)$ = the transport function of the reference substance (the non-diffusible tracer) ($h_1(t)$), $h_2(t)$ = the transport function of the test substance (the diffusible tracer) ($h_2(t)$).

(2) Capillary permeability surface area product

$$(PS_{\text{cap}}) = -f \cdot \ln(1 - E_{\text{max}}), \text{ where } f = \text{liver perfusion flow (ml/g min)}.$$

(3) Mean transit time

$$(MTT) = \frac{\int_0^\infty t h_1(t) dt}{\int_0^\infty h_1(t) dt}$$

where t = time (s), $h_1(t)$ = the transport function ($h_1(t)$).

(4) Distribution volume of the tracer concerned (V) = $f \cdot MTT$ (f of the total volume of the liver tissue). The total volume of the liver tissue was calculated from the weight of liver (Goresky 1963).

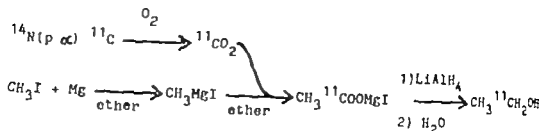
(5) Distribution coefficient

$$(D) = \frac{V - V_{\text{ref}}}{V_{\text{ref}}}$$

where V = distribution volume of test substance, V_{ref} = distribution volume of reference substance.

RESULTS

Responses to 3° injections of the double tracer solution containing ^{51}Cr -ethanol and ^{51}Cr -labelled red cells were obtained in 7 isolated rat livers. During one perfusion expt. 3-5 injections were carried out. The duration of the perfusion expt. varied from 30 to 45 min depending on the number of ethanol injections. The oxygen consumption of the perfused liver was constant for at least 90 min from the beginning of the expt. The mean consumption was $0.5 \pm 0.26 \mu\text{mol/g} \times \text{min}^{-1}$ ($N = 5$ 4 samples per rat were taken 20-90 min after the perfusion was begun). Two typical runs are shown in Fig. 4 following the single bolus injection the transport functions $h_1(t)$ and $h_2(t)$ were recorded over the outflow catheter and the instantaneous extraction ($E(t)$) calculated from equation (1). The appearance and mean transit times of reference and test substances were much longer at low flow rates. The

Fig 1 Scheme of the synthesis of 1-[^{11}C]-ethanol

distribution kinetics of ethanol in the isolated perfused liver at various flow rates and concentrations by the indicator diffusion technique (Chinard et al 1955; Crone 1963)

MATERIALS AND METHODS

The synthesis of 1-[^{11}C]-ethanol

C was produced by the $^{14}\text{N}(\text{p}, \alpha)^{11}\text{C}$ reaction using a gas target containing N_2 with less than 0.5% O_2 . The irradiations were carried out at the 103 cm cyclotron of Åbo Akademi, Turku (18 MeV protons, external beam of 50 μA current). In a typical run ^{11}CO was obtained in a yield of 200 mCi/0.5 h with a 1 MeV beam of protons of 10 μA current.

The synthesis of 1-[^{11}C]-ethanol was performed by a procedure earlier used (Raichle et al 1976) slightly modified according to Fig 1. The produced ^{11}CO was trapped in 1.5 ml of a 0.5 mol/l methyl magnesium iodide solution in ether (the Grignard solution was freshly prepared). After the trapping the solvent was evaporated and the residue was reduced with 3 ml of lithium aluminium hydride in ether (7 mg/ml). After another evaporation the residue was hydrolysed with water. The ethanol was trapped on transfer in a glass vial cooled with liquid N₂.

The radiochemical and chemical purity was analysed by radio-gas chromatography. The analysis was performed with a 1.5 m Porapak Q column (80–100 mesh) on an Aerograph 600-C gas chromatograph (Wilkens Instr. Res. Inc.). The radioactivity was detected with a NaI(Tl) scintillation detector. Nitrogen was used as carrier gas with a flow rate of 70 ml/min. The analysis was carried out at a temperature of 135°C.

More than 90% of the analysed activity was found in the ethanol peak, the rest found in the methanol peak. The time used for the synthesis starting from $^{11}\text{CO}_2$ was 20 min including analysis. The total yield of 1-[^{11}C]-ethanol was 50% corrected for decay. The use of carefully dried equipment throughout the synthesis was essential to avoid formation of impurities.

Perfusion experiments

Male Wistar rats (age 3–4 months) were used in the perfusion experiments. The mean weight was 400 g (range 360–430 g). The open perfusion system designed according to Hems et al (1966) is shown in Fig. 2. The perfusion pressure was regulated by changing liquid level. The Krebs bicarbonate perfusion buffer (Alabaster & Bakhle

1970) was continuously bubbled with carbogen gas (95% O_2 and 5% CO_2). The rat was given 0.2 ml heparin (10 IU/ml) and anaesthetized with ether. The abdomen was opened and cannulae inserted into the portal vein and cava inferior. The vena portae cannula was then connected to the perfusion system and the vena cava cannula to the outflow. The liver was perfused for 10–30 min before injections. The perfusion pressure and flow rate were measured. The tracer mixture (0.2 ml) consisting of about 50 μCi 1-[^{11}C]-ethanol and about 100 μCi ^{99}Tc -labelled red blood cells (^{99}Tc -RBC) in physiological saline was injected according to Bardy et al (1975). A bolus injection was given into the inflow tubing close to the liver. The wet content of liver tissue was calculated from the difference of wet and dry weight.

The viability and function of the perfused liver was tested by determinations of pO_2 , pCO_2 and pH of perfusion fluid from inflow and outflow tubings.

Measurement of radioactivity

The radioactivities of ^{99}Tc -labelled red blood cells

1-[^{11}C]-ethanol were monitored over the outflow catheter with a NaI(Tl) scintillation detector at a distance of 15 cm from the liver. The signal from the detector was taken through

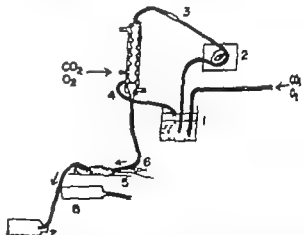


Fig 2 Schematic diagram of the perfusion system. (1) Buffer reservoir (at 37°C bubbled with carbogen) (2) roller pump (3) filter (4) oxygenator (at 37°C also bubbled with carbogen) It is an open system where the height of the liquid level determines the perfusion pressure (5) platform for rat (6) injection site (7) bottle for collecting outflow (8) NaI(Tl) detector

1. Liver (right) extraction and distribution volumes of ^{99m}Tc -red cells and ^{14}C -ethanol perfusion experiment

Results are explained in the text

Experiment	Liver weight (g)	E_{max}	V (%)	V (%)	γ
Tracer: about addition of radioactive ethanol	13.4 ± 0.3	0.95 ± 0.01	18.7 ± 0.5	56 ± 9.0	0.9 ± 0.75
Tracer: addition of non radioactive ethanol	13.4 ± 0.3	0.95 ± 0.01	19.0 ± 0.6	57 ± 9.9	0.4 ± 0.54

perfusion stops on 7 animals.
perfusion stops on 3 animals

1. Effect of flow and concentration on ethanol extraction and capillary permeability—area product

In experiments the flow of perfusion fluid ranged from $0.67 \text{ ml/g} \times \text{min}$ to $2.55 \text{ ml/g} \times \text{min}$. In this range E_{max} remained constant (0.95 ± 0.01) indicating that flow does not influence the maximal ethanol extraction (Table 1). The shortening of the plateau phase of the instantaneous extraction curve (Fig. 1) can be explained by faster back diffusion of ethanol from liver tissue to extravascular space with higher flow rate.

In some experiments non radioactive ethanol was added to the perfusion fluid to a final concentration $0.38 \pm 0.07\%$. This increase in the concentration of ethanol did not change the maximal extraction (Table 1). The radioactivity of the liver was followed by means of a separate gamma-ray counter externally in 11 expts to determine the amount of tracer which remained in the liver. The mean fraction of ^{14}C -radioactivity remaining in the liver after single passage was 6% (range 2–8%) of the injected dose. At high flow rates ($>2 \text{ ml/g} \times \text{min}$) the fraction of ^{14}C -radioactivity remaining in the liver was smaller (2%) than at low rates ($<1 \text{ ml/g} \times \text{min}$) (8%). This fraction is not detected, when radioactivity is measured above the outflow line. In calculations it should be taken into account.

The relation between the flow rate and capillary permeability surface area product is shown in Fig. 2. Value of PS_{cap} increases linearly as a function of flow rate (r). The equation describing the regression line is $PS_{\text{cap}} = 3.0 \pm 0.1 \times f$ ($r = 0.98$). The calculated PS_{cap} values ranged from $1.76 \text{ ml/g} \times \text{min}$ at low flow rates ($f = 0.67 \text{ ml/g} \times \text{min}$) to $7.63 \text{ ml/g} \times \text{min}$ at high flow rates ($f = 2.55 \text{ ml/g} \times \text{min}$).

Taking the mean capillary surface area S in the liver as $50 \text{ cm}^2/\text{g}$ (Crone 1963) the permeability coefficient of ethanol P_{mem} can be estimated giving the mean value $0.33 \pm 0.04 \times 10^{-4} \text{ cm}^2/\text{s}$ ($0.67 < f < 2.55 \text{ ml/g} \times \text{min}^{-1}$). The values of PS_{cap} in 7 expts with higher concentration of ethanol (addition of non radioactive ethanol) ranged from $3.6 \text{ ml/g} \times \text{min}$ at low rates ($f = 1.14 \text{ ml/g} \times \text{min}$) to $6.15 \text{ ml/g} \times \text{min}$ at high flow rates ($f = 1.5 \text{ ml/g} \times \text{min}$). Mean P_{mem} with higher ethanol concentration was $2.88 \pm 0.97 \times 10^{-4} \text{ cm}^2/\text{s}$.

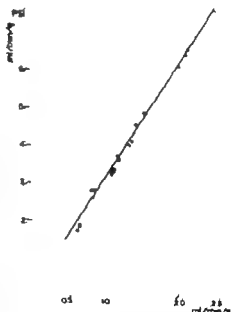


Fig. 5 Effect of flow on capillary permeability of ethanol. PS_{cap} rises linearly as a function of flow indicating flow limited distribution for ethanol in the liver. Symbols are defined in the text.

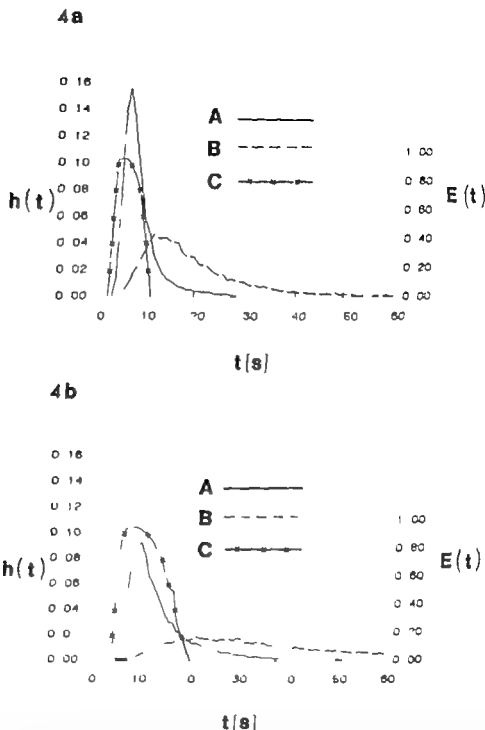


Fig. 4 Time-activity curves of ^{14}C -ethanol (the diffusible test substance) and $^{99\text{m}}\text{Tc}$ -red blood cells (the non-diffusible reference substance) were recorded by continuous detection of radioactivity in the venous outflow. The transport function and the instantaneous extraction of ethanol are shown at two different flow rates $f = 15 \text{ ml/g} \times \text{min}$ (Fig. 4a) and $f = 0.91 \text{ ml/g} \times \text{min}$ (Fig. 4b). A $^{99\text{m}}\text{Tc}$ -red blood cells, B ^{14}C -ethanol, C instantaneous extraction $E(t)$.

outflow radioactivity pattern of the fast diffusible ethanol shows a lower maximal concentration and longer transit time than that of red cells, which reflects the different volumes in which ethanol and red blood cells distribute in the liver tissue. The

instantaneous extraction of ethanol to the liver tissue is a fast process and the back diffusion of ethanol affects the descending part of the extraction curve. With equal values for $h(t)$ and $h_0(t)$ the instantaneous extraction becomes zero.

1. Liver eight extractions and distribution volumes of ^{125}I -red cell and C-ethanol in perfusion
 in all
 values are explained in the text

Experiment	Liver weight (g)	E_{max}	V (%)	V (%)	γ
repeated without addition of radioactive ethanol	13.4 ± 0.3	0.95 ± 0.01	18.7 ± 5	46.1 ± 9.0	0.9 ± 0.75
repeated with addition of radioactive ethanol	13.4 ± 0.3	0.95 ± 0.01	19 ± 6	57.3 ± 9	2.04 ± 0.54

perfusion experiments on 7 animals.
 reference experiments on 3 animals.

1. flow and concentration on ethanol action and capillary permeability— area product

Experiments the flow of perfusion fluid ranged from 0.67 ml/g × min to 2.55 ml/g × min. In this range E_{max} remained constant (0.95 ± 0.01) indicating that flow does not influence the maximal ethanol extraction (Table 1). The shortening of the plateau phase of the instantaneous extraction curve (Fig. 1) can be explained by faster back diffusion of ethanol from liver tissue to intra-vascular space with higher flow rate.

In some experiments non-radioactive ethanol was added to the perfusion fluid to a final concentration 0.34 ± 0.02%. This increase in the concentration of ethanol did not change the maximal extraction (Table 1). The radioactivity of the liver was followed by means of a separate gamma-ray monitor externally in 12 experiments to determine the amount of tracer which remained in the liver. The mean fraction of C-radioactivity remaining in the liver after a single passage was 6% (range 4–8%) of the injected dose. At high flow rates (>2 ml/g × min) the fraction of C-radioactivity remaining in the liver was smaller (7%) than at low rates (<1 ml/g × min) (8%). This fraction is not detected when radioactivity is measured above the outflow tubing. In calculations it should be taken into account.

The relation between the flow rate and capillary permeability surface area product is shown in Fig. 2. Value of PS_{eth} increases linearly as a function of flow rate (f). The equation describing the regression line is $PS_{\text{eth}} = 3.0 \pm 0.1 \times f$ ($r = 0.98$). The calculated PS_{eth} values ranged from 1.76 ml/g × min at low flow rates (f = 0.67 ml/g × min) to 7.63 ml/g × min at high flow rates (f = 2.55 ml/g × min).

Taking the mean capillary surface area S in the liver as 150 cm²/g (Cronk 1963) the permeability coefficient of ethanol P_{eth} can be estimated giving the mean value $1.83 \pm 0.05 \times 10^{-4}$ cm × s (0.67 < f < 2.55 ml/g × min⁻¹). The values of PS_{eth} in 7 experiments with higher concentration of ethanol (addition of non-radioactive ethanol) ranged from 3.6 ml/g × min at low rates (f = 1.14 ml/g × min) to 6.15 ml/g × min at high flow rates (f = 1.5 ml/g × min). Mean P_{eth} with higher ethanol concentration was $2.88 \pm 0.97 \times 10^{-4}$ cm × s.

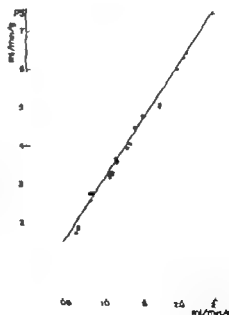


Fig. 3. Effect of flow on capillary permeability of ethanol. PS_{eth} rises linearly as a function of flow indicating flow limited distribution for ethanol in the liver. Symbols are defined in the text.

Volumes of distribution and distribution coefficient γ

The mean values and standard deviations of the distribution volumes for ethanol and red blood cells are presented in Table 1. The mean vascular distribution volume (V_1) calculated from the red blood cell curve was $18.7 \pm 2.5\%$ of the total liver volume and the mean distribution volume of ethanol was $56.2 \pm 9.0\%$ of the total liver volume in cases without addition of non-radioactive ethanol. The water content in isolated perfused livers after the experiment was determined in 3 experiments and was found to be 67% (range 65–69%).

The distribution coefficient γ which describes the relative distribution volumes of ethanol and red blood cells was 2.1 ± 0.7 . It was used to test if some factor influences the distribution volume of the test substance. The results indicate that variations of flow rate and ethanol concentration did not influence γ but the standard deviation was quite large.

DISCUSSION

Extraction and capillary permeability—surface area product for ethanol

The purpose of this study was to investigate extraction and distribution kinetics of ethanol in the isolated perfused liver. The viability of liver during perfusion experiment was investigated by determination of oxygen consumption. A constant oxygen extraction for at least 90 min from the beginning of the experiment was found. This agrees well with previous oxygen consumption measurements in the isolated perfused rat liver e.g. (Salaspuro 1968, Ross 1977). On the basis of these observations the perfusion technique used was considered to be valid. The maximal extraction values describe the capacity of ethanol to penetrate the blood tissue barrier in the liver. The small amount of C-methanol present in the C-ethanol preparation as impurity was considered to influence the results very little because methanol permeates capillaries about as effectively as ethanol (Raichle et al. 1976). In the liver there are large intercellular fenestrations in the endothelial lining of capillaries between the sinusoidal cells and so all solutes, crystalloids and colloids ($M < 90,000$) may freely penetrate from intravascular space to the interstitial space of Disse (Bennet et al. 1959, Keinänen et al. 1979). The mean diameter of the space of Disse is only 2 μm (Goresky 1963) and the diffusion of a

solute over this distance from the plasma to liver cell may be considered instantaneous. The parenchymal cell membrane can be considered the main factor limiting permeability of C_2 in the liver. Parameters extraction value PS_{eth} product measure therefore the permeability of ethanol through the parenchymal cell membrane. The obtained value for E_{max} for ethanol (0.8) agrees well with previous calculations in the liver. Crone (1965) obtained 93% extraction and Raichle et al. (1976) 97% extraction ($f = 0.5 \text{ ml/g} \times \text{min}$). The accuracy of the instantaneous extraction values is diminished because of the back diffusion of fast diffusible ethanol which very soon lowers the extraction value (see Fig. 4 and Goresky et al. 1975). Crone's model for capillary permeability in the tissues does not take into account back diffusion and to correct this we used E_{max} value in calculations of PS_{eth} values instead of the perfusion extraction E_p (Bassingthwaite 1974). In complicated models of capillary permeability it is possible to separate the throughput component and back diffusion component (double barrier model, Goresky 1970) but their use is tedious because of complicated mathematical formulas. Other factors which can influence the instantaneous extraction value are the heterogeneity of blood flow and relatively faster axial intravascular diffusion of ethanol compared to red blood cells (Gulley et al. 1975).

Since the extraction of ethanol is constant at various flow rates in the liver and PS_{eth} is related to flow rate, the ethanol distribution in liver is flow limited as is the distribution of C_2 (Goresky 1963, Bravo & Yudilevich 1971, Ross et al. 1977). The permeability coefficient P_1 is the value $8.3 \times 10^{-4} \text{ cm/s}$ in experiments without addition of non-radioactive ethanol) is comparable to that previously calculated for labelled water in myocardial capillaries ($6 \times 10^{-4} \text{ cm/s}$, Rose et al. 1977). The permeability coefficient for labelled water in the brain has been estimated to be $1.9 \times 10^{-3} \text{ cm/s}$ (Eckling et al. 1974 and Paul et al. 1977 respectively). P_{eth} calculated according to Crone's model is slightly underestimated because the model does not take into account the back diffusion phenomenon. The PS_{eth} value for brain reported by Raichle et al. (1976) is of the same magnitude ($1.8 \text{ ml/g} \times \text{min}$, $f = 0.5 \text{ ml/g} \times \text{min}$) as PS_{eth} for liver with same perfusion flow. PS_{eth} values without and with addition of non-radioactive

of differ slightly but the difference is so small it may be considered to be insignificant.

Form of distribution and distribution kernel for ethanol

The distribution volume of red blood cells in the liver ($18.7 \pm 5\%$) was similar to previous findings (19%) (Bravo & Yudilevich 1971). There is free exchange between the ^{14}C -red blood cell tracers (3.5% in our experiments) and this penetrates the space of Disse. However the volume of space of Disse is a rather small fraction (3%) of total liver volume (Bravo & Yudilevich 1971; Wemen et al. 1979) and therefore its influence on the slight ethanol distribution volume $\pm 9.0\%$ (without addition of non radioactive model) agrees with the water distribution volume (6.7% Bravo & Yudilevich 1971) and with calculations of the water content of the isolated liver (67%) (investigation of the plot of C-ethanol curve on a semilogarithmic scale).

It seems that the ethanol washout from the liver is exponential which indicates that there are several compartments into which ethanol penetrates (space of Disse, the liver parenchymal cells, metabolized component). The fraction of the radioactivity remaining in the liver after each fraction (7-8%) causes some underestimation in calculation of ethanol distribution volume when rebreaths are repeated during an experiment, because mean transit time for ethanol decreases. The distribution coefficient γ is useful when the effect of certain factors on the distribution volume test substance is to be considered. In this study variations in either flow or ethanol concentration did not change ethanol distribution volume (table 1).

Extraction of ethanol during a single capillary passage in the liver has not been studied earlier. Results obtained for the capillary permeability of ethanol in the liver in this study are of same order as those described earlier for the brain. The increase in the concentration of ethanol did not have any direct effect on the permeability in the range used. Distribution of ethanol in the perfused liver was found to be flow limited. Ethanol was distributed to the distribution volume. The indicator diffusion technique as shown to be useful in the study of intercapillary exchange of ethanol in tissues. ^{14}C -ethanol has many advantages in the study of ethanol metabolism: e.g. regional distribution of ethanol

can easily be followed by external detection without taking blood samples. Because of the short half-life of the tracer the experiments may be repeated frequently. The radiation dose is small which makes possible pharmacokinetic studies also in humans.

Authors are grateful to Prof. M. Bräster, Ph.D. the head of the Åbo Akademi radioisotope project who organized the radiochemical work and provided the cyclotron of Åbo Akademi and the equipment of the Department of Physics, Åbo Akademi at our disposal. We owe our warmest thanks to Prof. E. Nordman, M.D. and P. Mäkelä, M.Sc. who provided us with the equipment of the Radiation Therapy Clinic (University Central Hospital, Turku). Authors appreciate the collaboration and fruitful discussion with H. Lundqvist, Ph.D. and M. S. Møller, M.Sc. who expressed our gratitude to L. Hovvén, M.Sc. for constructing electronic equipment and help in programming. Prof. Bräster, Prof. R. Särnäs, M.D. and Dr. H. Wendelin, M.D. gave us valuable constructive criticism during the preparation of the manuscript. M. Kerkänen is research fellow of the Finnish Academy. The investigation was supported by a grant from the Finnish Foundation for Alcohol Studies, Helsinki.

REFERENCES

- ÅKESSON C. 1974. Autoradiographic studies on the distribution of ^{14}C -2-ethanol and its nonradioactive metabolites in the pregnant mouse. *Arch. Int. Pharmacodyn.* **204**, 295-304.
- ALABASTER, V. A. & BAKHLE, Y. S. 1970. Removal of ^{14}C -tryptophan in the pulmonary circulation of rat isolated lung. *Br. J. Pharmacol.* **40**, 468-482.
- ANTHONISEN P. & CRONE, C. 1956. Transcapillary migration of ethanol in pulmonary circulation. *Acta Physiol. Scand.* **37**, 370-379.
- BARDY, A., FOUYE, H., GIBIN, R., DE TOVAR, G., PANNACIERE, C. & HEGESTIPPE, M. 1975. Technetium $^{99\text{m}}$ labelling by means of sodium pyrophosphate. Application to leucocytes and red blood cells. *J. Nucl. Med.* **16**, 435-437.
- BASSINGHTWAIGHTE J. B. 1966. Plasma indicator dispersion in arteries of the human leg. *Circ. Res.* **19**, 332-346.
- BASSINGHTWAIGHTE J. B. 1974. A concurrent flow model for extraction during intercapillary passage. *Circ. Res.* **35**, 483-503.
- BASSINGHTWAIGHTE J. B., KNOPP, T. J. & HAZELRIG, J. B. 1970. A concurrent flow model for capillary tissue exchanges. In: *Capillary permeability* (ed. C. Crone and N. Lassen), pp. 60-80. Munksgaard, Copenhagen.
- BENNET, H. S., LUFT, J. H. & HAMPTON, J. O. 1959. Morphological characteristics of vertebrate blood capillaries. *Amer. J. Physiol.* **196**, 381-390.
- BRAVO, L. R. & YUDILEVICH, D. C. 1971. Liver distribution and uptake of molecules studied by rapid indicator dilution technique. *Amer. J. Physiol.* **221**, 1449-1455.

- CHINARD F P VOSBURG O J & ENNS T 1955 Transcapillary exchange of water and other substances in certain organs of the dog. *Amer J Physiol* 183 211-234
- CRONE C 1963 Permeability of capillaries in various organs as determined by use of the indicator diffusion method. *Acta Physiol Scand* 58 292-305
- CRONE C 1965 The permeability of brain capillaries to nonelectrolytes. *Acta Physiol Scand* 64 407-417
- DE GRAZIA J A RODDEN A F TEREZI J D BUSICK D B & WALZ D R 1975 Radioisotopic studies of C -distribution in cats given 14C ethanol. *J Nucl Med* 16 73-76
- EICHLING J O RAICHLE M E GRUBB R C Jr & TER POGOSSIAN M M 1974 Evidence of the limitations of water as a freely diffusible tracer in brain in the rhesus monkey. *Circ Res* 35 358-364
- ERICKSSON C A 1976 Regional distribution of ethanol in rat brain. *Life Sciences* 19 1439-1446
- FORSANDER O RÄIHA N & SUOMALAINEN H 1960 Oxydation des Äthylalkohols in isolierter Leber und isolierten Hinterkörper der Ratte. *Z Physiol Chem* 318 1-5
- GORDON E R 1966 Effect of aeration on the consumption of ethanol by the isolated perfused rat liver. *Nature* 209 1028-1029
- GORESKY C A 1963 A linear method for determining liver sinusoidal and extravascular volumes. *Amer J Physiol* 204 646-649
- GORESKY C A ZIEGLER W H & BACH G O 1970 Capillary exchange modelling. Barrier limited and flow-limited distribution. *Circ Res* 27 739-764
- GULLER B YIPINTSOI T ORVIS A L & BAS-SINGHTWAIGHT J B 1975 Myocardial sodium extraction at varied coronary flows in the dog. Estimation of capillary permeability by residual and out flow detection. *Circ Res* 37 359-378
- HEMS R ROSS B D BERRY M N & KREBS H A 1966 Gluconeogenesis in the perfused rat liver. *Biochem J* 101 284-29
- KEINÄNEN M KUIKKA J HESELIUS S J SUOLINNA E M SOLIN O MAKELA P HALKOLA L & NANTÖ V 1979 Carbon 11 labelled ethanol distribution in perfused rat liver. Proceedings of the Annual Conference of the Finnish Physical Society -3 Feb 1979 Jyväskylä
- KULONEN E & FORSANDER O 1969 ^{14}C products from labelled ethanol. I. Experimental autoradiography. *Arch Int Pharmacodyn* 170 1-7
- PAULSON O B HERTZ M M BOLWIG T L LASSEN N A 1977 Water filtration and flow across the blood brain barrier in man. *Acta Scand* 6 Suppl 5 49-493
- POE N D ROBINSON G D & MACDONALD N S 1973 Myocardial extraction of variously labelled acid and carboxylates. *J Nucl Med* 14 430
- RAICHLE M E, EICHLING J O STRAATM M G WELCH M J LARSSON K B & TER POGOSSIAN M M 1976 Blood-brain barrier permeability of C -labelled alcohols and ^{14}O labelled water. *Amer J Physiol* 30 541-551
- ROBINSON G D & MACDONALD N S 1973 Biological dynamics of ^{14}F and ^{14}C -labelled hydroxylates and alcohol. *J Nucl Med* 14 486-497
- ROSE C P GORESKY C A & BACH G O 1963 The capillary and sarcolemmal barriers in the liver. An exploration of labelled water permeability. *Circ Res* 41 515-533
- ROSS B D 1977 Perfusion techniques in biochemistry pp 193-213 Clarendon Press Oxford
- SALASPURO M P 1968 Studies on the metabolism of ethanol on the metabolism of the normal and pathological liver. Thesis Helsinki
- SCHERRER ETIENNE M & POSTERNAK I 1963 Penetration and distribution of ethanol and ^{14}C -barbiturate in rat brain. *Autoradiographic studies*. *Schweiz Med Wochenschr* 91 1016-1020
- TILBURY R S & LAUGHLIN J S 1974 C -labelled production of radioactive isotopes for medical research. *Seminars in Nuclear Medicine* 4 45-53
- WALLGREN H & BARRY H 1970 Actions of alcohol. pp 16-63 Elsevier Amsterdam
- WIDMARK E M P 1937 Die theoretischen Grundlagen und die praktische Verwendbarkeit der physikalisch-medizinischen Alkoholbestimmung (ed. E. Widmark). Heft 11 Urban und Schwarzenberg, Berlin
- WOLF A P & REDVANDLY C S 1977 Carbon 11 and radiopharmaceutical. *Int J Appl Rad Isot* 3 48

Distension of pulmonary vein—left atrial junction Heart rate responses in conscious and anesthetized dogs

NEVALAINEN M O K HAKUMÄKI S J HYÖDYNNÄÄ
O NÄRHI and H S S SARAJAS

Department of Physiology, University of Kuopio and College of Veterinary Medicine,
Kuopio, Finland

NEVALAINEN T O HAKUMÄKI M O K HYÖDYNNÄÄ S J NÄRHI M V
O & SARAJAS H S S Distension of pulmonary vein—left atrial junction: Heart rate
responses in conscious and anesthetized dogs. *Acta Physiol Scand* 1980 110: 47-52. R
ceived 23 Nov 1979 ISSN 0001-6772. Department of Physiology, University of Kuopio
and College of Veterinary Medicine, Helsinki, Finland

The cardiac chronotropic effects of distension of pulmonary vein—left atrial junction were
investigated in conscious dogs and in dogs anesthetized with intravenous alpha-chloralose
(100 mg/kg) or pentobarbital (30 mg/kg). All the experiments were made on trained,
chronically instrumented, closed chest animals held in horizontal position. Inflation of
single small balloon in the junction elicited tachycardic response both in conscious and
chloralose anesthetized dogs, while in pentobarbital anesthesia no change in heart rate was
found. Contrary to reports of other investigators, no transient bradycardia was found nor
any correlation between pre-distension heart rate and the increase in heart rate due to the
distension. It is suggested that the chance of anesthesia can be done only after its modifying
effect on the parameter studied is known. Only the use of trained, chronically instrumented
and conscious dogs will reveal this effect.

Key words: Mechanoreceptors, heart stretch, heart rate, consciousness, chloralose, pen-
tobarbital, dogs.

Reflex tachycardia has been found to result from
distension of the pulmonary vein—left atrial
junction in anesthetized dogs (Ledsome & Linden
& Edm et al 1970; Kollai et al 1978; Chapman
et al 1978). The afferent pathway of this reflex is
found to be in the vagus (Ledsome & Linden
et al) and the efferent pathway both in sympathetic
and parasympathetic nerves (Kollai et al 1978;
Epstein et al 1978).

Like all the studies involving this reflex have
been done on anesthetized animals, and all but one
working groups have used open chest animals
(Epstein et al 1978). Yet there is growing evi-
dence that anesthetics variously affect the car-
diovascular functions and their neural control
(Mist & Page 1966; Epstein et al 1968; Horvatz
et al 1969; Sawyer et al 1971; Vainer & Braunwald
1975; Hatanaka et al 1978). Furthermore opening of
thorax is known to influence the cardiovascular
parameters including the cardiodynamics and the
movements of the heart (Ferguson et al 1953;

Rushmer et al 1954; Morgan et al 1966; Giles &
Bryce 1970). To the best of our knowledge the
effects of the distension of the pulmonary vein—left
atrial junction have not been studied in conscious
and closed chest animals. The present study was
designed to compare the heart rate changes elicited
by the distension in conscious dogs to those in dogs
anesthetized with chloralose or pentobarbital.

MATERIALS AND METHODS

Animals

Eight young laboratory-bred beagle dogs of both sexes,
weighing 8-10 kg, were used in this study.

Surgical procedures

Prior to the surgical procedure the dogs were accustomed
to a restraining harness and laboratory surroundings dur-
ing 3-4 consecutive days. A sterile left thoracotomy was
performed under pentobarbital anesthesia and positive
pressure ventilation. A thrombolectomy balloon catheter
(capacity 2.5 ml) was passed via the pulmonary vein of the
left apical lobe into the junction of the caudal left atrium

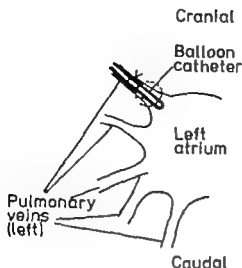


Fig. 1. Dorsoventral silhouette of canine left atrium showing left pulmonary vein-left atrial junctions. A thrombolectomy balloon catheter was passed via the pulmonary vein of the left apical lobe into the junction in such a manner that the tip of the catheter just entered left atrium. Dotted line illustrates the silhouette of the inflated balloon.

A more detailed location of the catheter is illustrated in Fig. 1. The catheter was fixed in place with ligatures around the pulmonary vein and the left apical lobe of the lung was subsequently removed. The catheter was passed out of the thorax and carried under the skin to the neck where it was placed under a protective collar. The dogs were allowed to recover for 3-4 days after the surgery before the distension experiment.

Experimental protocol

The distension experiments were performed on dogs fasted overnight and kept in restraining harnesses (Sline & Englert Jr 1965) in a natural horizontal position slightly off the floor. The experiments took place in an isolated laboratory where external stimuli could be excluded. The ECG signal was recorded telemetrically with needle elec-

trodes and stored during the experiment on an interval tape recorder (PI-6200). The distension period when the balloon was inflated, was preceded by control periods of equal length (3 min) in conscious state the dogs were allowed to bury harness for about 10-15 min to calm down before commencement of the experiment. Alpha-chloralose (mg/kg) or solution Alpha-D(+)-gluco-chloralose (Merck) or pentobarbital (30 mg/kg Nembutal, a) was given intravenously via the cephalic vein 2-4 min prior to the experiment. After experiments were completed they were anesthetized they were allowed to recover at least 2 days before the next distension was carried out.

Data processing

The heart rate was calculated for 15-s periods (HR) RR intervals of ECG

$$HR = \frac{60s}{n \cdot T_i}$$

Where n = number of complete RR-intervals during T_i = duration of individual RR interval (s).

The lengths of individual RR interval were measured by the timing unit of a multichannel analyser (Nihon Kohden 4900) with the accuracy of 1 ms. The heart rate change (ΔHR) was calculated for every 15-second period. The distension of the balloon and for the first 15 s after the deflation of the balloon. The linear estimates of ΔHR if no change had been induced for the period were calculated using the HR values of the 15 min of the control periods both before and after the distension period. This method of calculation is illustrated in an example in Fig. 2. The aim of this procedure is to eliminate the changing trends in heart rate observable in a few of the experiments presumably due to the depth of anaesthesia.

The mean heart rate change and standard error of mean were calculated from pooled data for every 15-min interval in conscious animal and those given chloralose or pentobarbital. The statistical significance of changes was tested using the paired Student's t -test. Furthermore the mean heart rate within each period

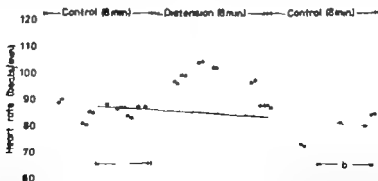


Fig. 2. Illustration of the method used to calculate heart rate changes (ΔHR) in one experiment. The latter half of both control period (a and b) were used to calculate linear estimates of HR_{15} (line) if no change had been induced. Utilizing this estimate ΔHR_{15} value were processed for the distension period and the first half of the control period after distension.

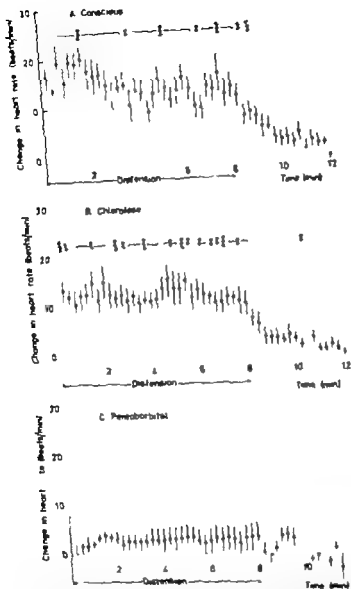


Fig. 3. Effect of distension of pulmonary vein-left atrial junction on heart rate change (ΔHR_{L_1}) in conscious dogs (A) and in those anesthetized with alpha-chloralose (B) or pentobarbital (C). Vertical lines indicate standard error of mean (S.E.M.). Significance symbols: $***P<0.001$, $**P<0.01$ and $*P<0.05$. The pre-distension heart rates are 102.3 ± 18.6 per min (mean \pm S.D.) for conscious, 87.5 ± 7.8 per min for chloralose anesthetized and 70.1 ± 7.5 per min for pentobarbital anesthetized dogs.

baseline (initial heart rate) and the mean heart rate were within 2 min after the distension of the balloon re-inflated for correlation.

RESULTS

Results from 25 distensions (average ΔHR_{L_1}) in conscious dogs are shown in Fig. 3 A. Distension of the

pulmonary vein-left atrial junction elicited a tachycardic response which persisted through the whole distension period of 8 min. An increase in the heart rate was evident already during the first 15 s and maximal tachycardia was observed at 1 min after inflation of the balloon. The tachycardic response vanished within 1 min after deflation of the

balloon. The heart rate immediately before inflation of the balloon was 107.3 ± 18.6 per min (mean \pm S D) in experiments with conscious animals. The correlation coefficient calculated between the initial heart rate and the mean heart rate increase within the first 2 min after distension was 0.17 ($n=25$ N S).

Fig. 3B presents the data from 8 distensions in dogs anesthetized with chloralose. Again a tachycardic response resulted from the distension of the pulmonary vein-left atrial junction. The response was observable within the first 15 s and persisted all through the distension period. After deflation of the balloon the tachycardic response disappeared within 1 minute. The dogs with chloralose anesthesia had a predistension heart rate of 87.5 ± 7.8 per min (mean \pm S D). The correlation coefficient calculated between the initial heart rate and the mean heart rate increase within the first 2 min after distension was 0.10 ($n=8$ N S).

In dogs anesthetized with pentobarbital the distension of the pulmonary vein-left atrial junction produced no significant changes in heart rate. The results of 7 distensions under pentobarbital anesthesia are shown in Fig. 3C. The pentobarbitalized dogs had a heart rate of 170.1 ± 7.5 per min (mean \pm S D) just prior to the distension. The correlation coefficient calculated between the initial heart rate and the mean heart rate change within the first 2 min after distension was 0.70 ($n=7$ N S).

DISCUSSION

The heart rate responses to distension of left atrial receptors are reportedly controversial. Stimulation of these mechanoreceptors has been found to elicit bradycardia (Daly et al 1937; Douthett & Kramer 1959) or to leave the heart rate unchanged (Daly et al 1937; Aviador & Schmidt 1959). According to Ledson & Linden (1964) the overall response is tachycardia. Consonant with this are the results of several subsequent groups (Edis et al 1970; Koizumi et al 1975; Chapman et al 1978; Kollai et al 1978). However, even these more recent studies disagree among other things as to the initial effects of the distension on the heart rate and as to whether there is a correlation between the predistension heart rate and the degree of tachycardia attained.

These controversial results may emanate from experimental variables, especially from the use and depth of anesthesia. The most commonly used

anesthetics in physiological research are pentobarbital and chloralose. However, the impact of compounds on the results and their interpretation is commonly disregarded. This may be due to the fact that the circulatory and autonomic effects of anesthetics are poorly known (Manders & 1976) and that their direct and indirect effects are hard to differentiate from each other (Bir 1977).

Pentobarbital is known to produce tachycardia (Van Citters et al 1964), presumably owing to its vagolytic action (Morrison et al 1960; Van Pardo 1967; Page & Hoff 1969). Furthermore, its effects on the autonomic nervous system are heterogeneous and wide alterations in the rate-dependent variables are essentially eliminated by pentobarbital (Van Citters et al 1964).

Chloralose again is widely considered an anesthetic of choice in studies on circulation control. Yet even this compound modulates the cardiovascular and autonomic functions. It has been reported to increase the heart rate and arterial pressure (Bass & Buckley 1966). Furthermore, it also disturbs autonomic reflexes in an unpredictable fashion (Cron 1964). Mechanoreceptor reflexes controlling the heart rate, for example, appear to be exaggerated under chloralose (Cox 1977).

To overcome complications produced by anesthesia we carried out a series of distensions in conscious animals. According to our results, tachycardic response to the distension of the pulmonary vein-left atrial junction can be elicited in trained conscious dogs in circumstances where external stimuli have been minimized.

When chloralose was used for anesthesia there was a tachycardic response to the distension, similar to that seen in conscious dogs. A distension-induced tachycardia has been previously reported in dogs under chloralose anesthesia (Ledson & Linden 1964; Edis et al 1970; Kollai et al 1975; Kollai et al 1978; Chapman et al 1978). Only Edis et al (1970) and Kollai et al (1978) have paid attention to the time course of heart rate changes during the distension. Edis et al report that tachycardia was the response to distension only in those dogs with an initial (predistension) heart rate below 140 per min, whereas in those with initial heart rates over 140 per min there was initially bradycardia lasting less than 1 min. Subsequently there was no further change. Kollai et al in turn reported that the stretch of the k

the pulmonary vein region decreases the heart rate during the first 15 s following which a slight acceleration supervenes. We were not able to confirm these findings of Edis and Kollai's (1978).

There was no correlation between the initial heart rate and the heart rate increments in our anaesthetized or conscious dogs. However, in neither group did we encounter initial heart rates as high as did Edis and Kollai's groups in their anesthetized animals. Actually, in no experimental session of the present study did the initial heart rate come even close to the basal value of 140 per min. This difference may be traceable to the fact we used plain chloralose in a somewhat higher (than did the other groups, who besides three dogs morphine for premedication and thiopental induction of anaesthesia).

In this study we used 15 s intervals to calculate heart rate. This interval should be long enough to minimize the effect of sinus arrhythmia frequently encountered in dogs. If the initial bradycardic response to the distension was real, as suggested by Liu et al. (1978), it should be seen in pooled data obtained for the first 15 s after inflation of the balloon (Fig. 3A for conscious and Fig. 3B for anaesthetized dogs). According to Kollai et al. the initial bradycardia occurred during the first 20 s after the stretch was begun. However, as seen from Fig. 3A and B, there was a highly significant increase in heart rate during the first 15 s for conscious and chloralose dogs. Indeed, the averaging through individual distensions shows that only one out of 25 distensions was there a decrease in heart rate in the conscious state, while all distensions under chloralose anaesthesia resulted in increased heart rate at this interval. Kollai et al. noted that in experiments where only acceleration observed large doses of chloralose were used, rather large doses of chloralose. Yet the fact that the initial cardiac deceleration was not detectable in our experiments is scarcely attributable to this. This is evidenced by the comparable heart rate responses in our conscious dogs free of any pharmacological intervention.

The discrepancy could be explained through the effect of morphine, which has been shown to have potent effects even at small dose levels (Page & Off 1969). In the experiments presented here we did not use morphine as did the two other groups (Liu et al. 1970; Kollai et al. 1978). In dogs that had

received only morphine premedication (1 mg/kg) distension failed to increase the heart rate (own unpublished observation).

In pentobarbitalized dogs distension of the pulmonary vein-left atrial junction did not have any effect on heart rate as shown in Fig. 3C. This could be explained through the vagal blocking effect of pentobarbital. Besides this vagolytic action, pentobarbital appears to have also sympatholytic properties (Haltinen et al. 1978).

A wide variety of methods have been used to stimulate left atrial receptors. They include use to several balloons variously placed within or outside of the pulmonary vein-left atrial junction (Ledsome & Linden 1964; Edis et al. 1970; Kollai et al. 1973; Chapman et al. 1978). Others have used threads combined with a pulley and weights (Kollai et al. 1978). The use of several small balloons placed into the junction through pulmonary veins necessitates the ligation of all these veins (Ledsome & Linden 1964). This implies that practically no blood enters the left atrium from the left side of the lung.

A larger single balloon as used by Chapman et al. (1978) partially obstructs mitral valve and blood flow. The use of threads and pulleys necessitates the use of the open thorax. We used only one balloon placed in the junction of the left atrium and the pulmonary vein of the left apical lobe. This necessitated the removal of only one lobe, the apical, while preparing the animal. Our method of stimulating the receptors provides the least possible interference with blood flow with the possibility of using chronically instrumented closed chest animals. Our results also shows that one small balloon when correctly placed, produces sufficient stimulus for eliciting this cardioacceleratory reflex.

It appears that the distension of the pulmonary vein-left atrial junction evokes a tachycardic response, an effect that can be seen in unanesthetized conscious dogs and in dogs anesthetized with chloralose but not in dogs anesthetized with pentobarbital. No initial deceleration of the heart at the beginning of the distension was encountered. Nor was any correlation between the initial heart rate and the change in heart rate in any of the experimental states used.

We conclude that anaesthetics should be used with care unless their modifying effect on the reflex studied is known. Apparently the quality of this effect as well as the effect of other corresponding

balloon. The heart rate immediately before inflation of the balloon was 107.3 ± 18.6 per min (mean \pm S D) in experiments with conscious animals. The correlation coefficient calculated between the initial heart rate and the mean heart rate increase within the first 2 min after distension was 0.17 ($n=25$ N S).

Fig. 3B presents the data from 8 distensions in dogs anesthetized with chloralose. Again a tachycardic response resulted from the distension of the pulmonary vein-left atrial junction. The response was observable within the first 15 s and persisted all through the distension period. After deflation of the balloon the tachycardic response disappeared within 1 minute. The dogs with chloralose anesthesia had a predistension heart rate of 87.5 ± 7.8 per min (mean \pm S D). The correlation coefficient calculated between the initial heart rate and the mean heart rate increase within the first 2 min after distension was 0.10 ($n=8$ N S).

In dogs anesthetized with pentobarbital the distension of the pulmonary vein-left atrial junction produced no significant changes in heart rate. The results of 7 distensions under pentobarbital anesthesia are shown in Fig. 3C. The pentobarbitalized dogs had a heart rate of 170.1 ± 27.5 per min (mean \pm S D) just prior to the distension. The correlation coefficient calculated between the initial heart rate and the mean heart rate change within the first 2 min after distension was 0.70 ($n=7$ N S).

DISCUSSION

The heart rate responses to distension of left atrial receptors are reportedly controversial. Stimulation of these mechanoreceptors has been found to elicit bradycardia (Daly et al 1937, Douthett & Kramer 1959) or to leave the heart rate unchanged (Daly et al 1937, Aviador & Schmidt 1959). According to Ledsome & Linden (1964) the overall response is tachycardia. Consonant with this are the results of several subsequent groups (Edis et al 1970, Koizumi et al 1975, Chapman et al 1978, Kollai et al 1978). However, even these more recent studies disagree among other things as to the initial effects of the distension on the heart rate and as to whether there is a correlation between the predistension heart rate and the degree of tachycardia attained.

These controversial results may emanate from experimental variables, especially from the use and depth of anesthesia. The most commonly used

anesthetics in physiological research are peribital and chloralose. However, the impact of compounds on the results and their interpretation is commonly disregarded. This may be due to the fact that the circulatory and autonomic effects of anesthetics are poorly known (Alanders & 1976) and that their direct and indirect effects are hard to differentiate from each other (He 1977).

Pentobarbital is known to produce tachycardia (Van Citters et al 1964), presumably on a vagolytic action (Morrison et al 1940, Vah Pardo 1967, Page & Hoff 1969). Furthermore, spurious and wide alterations in the rate-dependent variables are essentially eliminated by pentobarbital (Van Citters et al 1964).

Chloralose again is widely considered an anesthetic of choice in studies on circulation control. Yet even this compound modulates the cardiovascular and autonomic functions. It has been reported to increase the heart rate and the arterial pressure (Bass & Buckley 1966). Furthermore, it also disturbs autonomic reflexes in an unpredictable fashion (Cronk 1962). Mechanoreceptor reflexes controlling the heart rate, for example, appear to be exaggerated by chloralose (Cox 1977).

To overcome complications produced by anesthesia we carried out a series of distensions in conscious animals. According to our results, tachycardic response to the distension of the pulmonary vein-left atrial junction can be elicited in trained conscious dogs in circumstances where external stimuli have been minimized.

When chloralose was used for anesthesia there was a tachycardic response to the distension, but similar to that seen in conscious dogs. A distension-induced tachycardia has been previously reported in dogs under chloralose anesthesia (Ledsome & Linden 1964, Edis et al 1970, Koizumi et al 1975, Kollai et al 1978, Chapman et al 1978). Only Edis et al (1970) and Kollai et al (1978) have paid attention to the time course of heart rate changes during the distension. Edis et al reported that tachycardia was the response to distension only in those dogs with an initial (predistension) heart rate below 140 per min, whereas in those with initial heart rates over 140 per min there was initial bradycardia lasting less than 1 min. Subsequently there was no further change. Kollai et al in turn reported that the stretch of the

Plasma water and ^{51}Cr EDTA equilibration volumes in different tissues in the rat

LARSSON L, JOHNSON G, NYLANDER U and ÖHMAN

Department of Surgery, Karolinska Hospital, Stockholm, Sweden

LARSSON L, JOHNSON G, NYLANDER U, ÖHMAN U. Plasma water and ^{51}Cr EDTA equilibration volumes of different tissues in the rat. *Acta Physiol Scand* 1980 110: 53-57. Received 28 Nov 1979. ISSN 0001-6772. Department of Surgery, Karolinska Hospital, Stockholm, Sweden.

The EDTA space and the plasma water volume of the whole animal and selected tissues were investigated with constant EDTA infusions of different duration in the rat. The calculated EDTA space increased with the duration of the infusion both in the whole animal and the tissues sampled. The plasma water volume remained constant, and therefore the calculated increase of the EDTA space suggests slow intracellular accumulation of the tracer. As extracellular accumulation of EDTA increases with experimental time it is necessary to use as short an experimental interval as possible to reduce this error when determining extracellular fluid volumes.

Key words: EDTA, extracellular space, isotopes, animal experiments.

The extracellular fluid volume is measured by tracer dilution techniques, where the marker diffuses throughout the extracellular fluid without getting into the cells. Such an ideal substance has not been found. EDTA has been chosen as a convenient substance for studies of the extracellular fluid volume (Bernard & Biron 1971; Boboe et al 1968; Brading & Jones 1969; Virgilio et al 1970; Joffe et al 1971). It is almost completely eliminated through the kidneys (Abrams & Aronson 1971; Rochoer-Mortensen et al 1969; Downes & McDonald 1964; Foreman et al 1953; Foreman & Trujillo 1954; Garnett et al 1967; Lachme et al 1976; Pripic 1967) with only an insignificant fraction being metabolized (Volf et al 1971; Foreman et al 1953; Foreman & Trujillo 1954; Havlicek et al 1968) or bound to plasma proteins (Bohne et al 1968; Volf et al 1971; Garnett et al 1967; Slacy & Borburn 1966). We have recently investigated EDTA tracer kinetics in the extracellular fluid in the rat (Larsson et al unpublished data). It was found that the rapid renal elimination of the tracer in this animal made space studies unreliable but when renal loss was excluded the EDTA space could be determined with more precision. This latter modification however involved surgical procedure which in itself might bias the results.

The aim of the present study was to estimate the extracellular fluid volume and the plasma water volume of different tissues in the rat using Cr EDTA and ^{125}I human serum albumin as tracers. A constant infusion of EDTA was used to compensate for renal losses and to achieve a stable concentration of the tracer. To test the hypothesis of a slow but steady intracellular penetration of EDTA we chose to vary the duration of the constant infusion.

METHODS

Male albino Sprague-Dawley rats weighing approximately 230 g were used. They were kept in the laboratory under standardized conditions for at least one week before the experiments. The animals fasted for 12-18 h prior to the experiments, but received water ad libitum.

The rats were anesthetized with Pentothal Sodium (Abbott) intraperitoneally 5.0 mg/100 g b.wt. The left carotid artery and the right jugular vein were cannulated with fine-bore catheters, the former for blood sampling and blood pressure registration (Siemens-Elema 746 pressure transducer and Siemens-Elema Magnograf 803 multi-channel writer) the latter for tracer injections.

A commercial solution of ^{51}Cr EDTA in isotonic saline (Behringwerke AG, Marburg) with a concentration of 37 MBq/ml was used. Of this solution 0.1 ml/100 g b.wt. was given as bolus dose followed by constant infusion (young pump model 35, Sage Instrument) with 35% of the bolus dose diluted to 1 ml and infused at a rate of 1

factors operant during most acute experiments e.g. open chest, side position and machined ventilation can be omitted only through the use of chronically instrumented, trained and conscious experimental animals.

This study was supported by the Academy of Finland and the North Savo Cultural Foundation.

REFERENCES

- AVIADO D M Jr & SCHMIDT C F 1959 Cardiovascular and respiratory reflexes from the left side of the heart. *Amer J Physiol* 196: 776-730.
- BASS B G & BUCKLEY N M 1966 Chloralose anesthesia in the dog: A study of drug actions and analytical methodology. *Amer J Physiol* 10: 854-86.
- CHAPMAN L W, MITTMAN U, PRICKETT C & RICHMOND W 1978 Left atrial receptor inhibition of cardiac efferent vagal activity in the dog. *Clin Exp Pharmacol Physiol* 5: 471-476.
- COX R H 1977 Influence of chloralose anesthesia on cardiovascular function in trained dogs. *Amer J Physiol* 233: 660-667.
- CROFT P G 1964 Actions and dosage of chloralose. *Nature* 203: 1046.
- DALY J de B, LUDANY G, TODD A & VERNEY E B 1937 Sensory receptors in the pulmonary vasculature. *Quart J Exp Physiol* 27: 123-146.
- DOUTHEIL U & KRAMER K 1959 Über die Differenzierung kreislaufregulierender Reflexe aus dem linken Herzen. *Mitg Arch Ges Physiol* 269: 114-119.
- EDIS A J, DONALD D E & SHLPHARD J T 1970 Cardiovascular reflexes from stretch of pulmonary vein-atrial junctions in the dog. *Circulat Res* 27: 1091-1100.
- EPSTEIN R A, WANG H & BARTELSTONE H J 1968 The effect of halothane on circulatory reflexes of the dog. *Anesth* 29: 867-876.
- FERGUSON T B, SHADLE O W & GREGG D E 1953 Effect of blood and saline infusion on intracardiac end diastolic pressure, stroke work, stroke volume and cardiac output in the open and closed chest dog. *Circulat Res* 1: 64-68.
- GILES T D & BURCH G E 1970 Anesthesia dogs and cardiovascular data. *Amer Heart J* 79: 141-14.
- HALINEN M O, HAKUMAKI M O K & SARAJAS H S N 1978 Suppression of autonomic postganglionic discharges by pentobarbital in dogs with or without endotoxaemia. *Acta Physiol Scand* 104: 167-174.
- HORWITZ L D 1977 Effect of intra-venous anesthetic agent on left ventricular function in dogs. *Amer J Physiol* 233: H44-H48.
- HORWITZ L D, BISHOP V S, STONE H J, STEGALL H F 1969 Cardiovascular effects of low-oxygen atmospheres in conscious and anesthetized dogs. *J Appl Physiol* 7: 370-377.
- KOIZUMI K, ISHIKAWA T, NISHINO T, BROOKS C McC 1974 Cardiac and autonomic reactions to stretch of the atria. *Br J* 87: 47-61.
- KOLLAI M, KOIZUMI K, YAMASHITA Y, BROOKS C McC 1978 Study of cardiac sympathetic and vagal efferent activity during reflexes produced by stretch of the atria. *Brain Res* 145: 53.
- LEDSONE J R & LINDEN R J 1964 A reflex increase in heart rate from distension of the pulmonary-atrial junctions. *J Physiol* 170: 456-477.
- MANDERS W T & VATNER S F 1976 Effects of sodium pentobarbital anesthesia on left ventricular function and distribution of cardiac output with particular reference to the mechanism of tachycardia. *Circulat Res* 39: 51-57.
- MORGAN B C, MARTIN W E, HORNBEIN T, CRAWFORD E W & CUNTHERO W G H 1969 Hemodynamic effects of intermittent positive pressure respiration. *Anesthesiol* 7: 584-590.
- MORRISON J L, WALKER H A & RICHARDS A P 1950 The effect of pentobarbital on the response of cardiovascular system of dogs to respiratory acetylcholine and tilting. *Arch Int Pharmacodyn* 53: 62.
- OLMSTED F & PAGE I H 1966 Hemodynamic changes in dogs caused by sodium pentobarbital anesthesia. *Amer J Physiol* 210: 817-820.
- PAGE C P & HOFF H E 1969 Cardiovascular respiratory relationships in the morphine-pentobarbital anesthetized dog. *Arch Int Pharmacodyn* 177: 331.
- RUSHMER R F, FINLAYSON H L & NASH L 1954 Shrinkage of the heart in anesthetized thoracotomized dogs. *Circulat Res* 2: 37-47.
- SAWYER D C, LUMB W V & STONE H L Jr 1964 Cardiovascular effects of halothane, methoxyflurane, pentobarbital and thiopental. *J Appl Physiol* 26: 641.
- SINE P N & ENGLERS E Jr 1965 Reversible jacket for prolonged experiments on dogs. *Lab J Care* 15: 45-455.
- VAN CITTERS R L, FRANKLIN D L & RUSMER R F 1964 Left ventricular dynamics in dogs during anesthesia with alpha-chloralose and sodium pentobarbital. *Amer J Cardiol* 13: 349-354.
- VATNER S F & BRAUNWALD E 1973 Cardiovascular mechanism in the conscious state. *New Eng J Med* 289: 970-976.
- VIDRIO H & PARDO E G 1967 Increased sympathetic vasoconstrictor tone in dogs treated chronically with mecamylamine. *J Pharmacol Exp Ther* 164: 4-48.

c Blood pressure (mmHg), plasma water volume (ml/100 g b. t.) and EDTA space (ml/100 g b. t.) in experimental group. Mean \pm S.D.

	Initial blood pressure	Final blood pressure	Plasma water volume	EDTA space
no infusion	13 \pm 16	128 \pm 19	3.65 \pm 0.55	24.4 \pm 6.7
60 min infusion	125 \pm 12	131 \pm 13	3.44 \pm 0.42	26.2 \pm 7.8
120 min infusion	177 \pm 13	122 \pm 14	3.42 \pm 0.57	28.6 \pm 7.0

(60 min \pm 120 min, $P < 0.05$)

Table 3 Absolute and relative method error in the determination of plasma water volume and EDTA space in liver and spleen

	Liver		Liver		Spleen	
	Plasma water volume	EDTA space	Plasma water volume	EDTA space	Plasma water volume	EDTA space
number of animals (3 samples per animal)	23	20	4	1	4	22
mean (all samples), μ l/g tissue	153	385	143	283	51	140
SD (all samples), μ l/g tissue	33	59	37	36	6.2	77
relative method error, μ l/g tissue	14	79	15	18	5	83
relative method error, %	9	7.5	10.5	6.4	4.9	5.9

When comparing the 120 min and 60 min groups, only the lung showed a significant reduction in its plasma water volume ($P < 0.01$).

The 90 min values of the EDTA space for all the tissues were greater than at 60 min although only the liver ($P < 0.05$) and the large intestine ($P < 0.01$) reached statistical significance.

At 120 min an increase of the EDTA space was noted for all the tissues except the skeletal muscle, failing to reach significance only in the aortal part of the stomach and the omental fat.

DISCUSSION

Intracellular accumulation of a tracer results in a higher calculated tracer space volume which is related to the experimental time. The EDTA space of the experimental animals tended to increase with the length of the experimental time, the 120 min value being 17% greater than the 60 min value. A probable explanation is that intracellular accumulation of EDTA has occurred in proportion to the time of exposure.

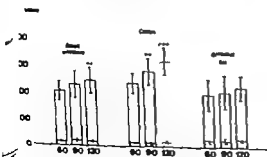


Fig. 3 Plasma water volume (hatched area) and EDTA space of small intestine, colon and omental fat after EDTA infusion of different duration. Mean \pm S.D. Significance as in Fig. 1.

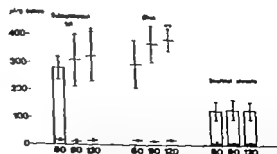
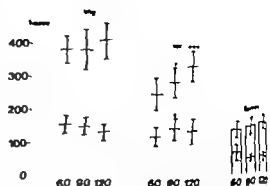


Fig. 4 Plasma water volume (hatched area) and EDTA space of subcutaneous fat, skin and skeletal muscle after EDTA infusion of different duration. Mean \pm S.D. Significance as in Fig. 1.

Table 1 Number and body weight in the experimental groups. Mean \pm S.D.

	n	Body weight (g)
60 min infusion	23	45 \pm 25
90 min infusion	1	61 \pm 39
120 min infusion	20	54 \pm 50

Fig. 1 Plasma water volume (hatched area) and EDTA space of lung, liver and spleen after EDTA infusion of different duration. Mean \pm S.D. Statistically significant differences are indicated ($P < 0.05$) ($P < 0.01$) ($P < 0.001$).

ml/h. Infusions of 60, 90 and 120 min were given. Three min before the end of the infusion period a bolus dose of 0.1 ml/100 g b.wt. of an 51 I human serum albumin solution (130 kBq/ml) was given i.v.

Immediately following the end of the EDTA infusion the animals were sacrificed and the following tissues sampled: lung, liver, spleen, gastric fundus and antrum, small and large intestine, omental and subcutaneous fat, skin and skeletal muscle. The tissue specimens were immediately weighed wet and thereafter counted in a gamma scintillation detector (Selektroil model 54-1). From a representative number of experiments selected tissues were sampled in triplicate enabling the error of the method to be calculated.

The following formula was used to calculate the tissue spaces of the tracers:

$$V = S_0/S_p$$

where V = volume of distribution in tissue (μ l/g), S_0 = concentration of tracer in whole tissue (μ g/g), S_p = concentration of tracer in plasma water (μ g/ μ l). The plasma water volume was attained from the plasma volume by using the factor 0.929 (Altman & Dittmer 1961).

The EDTA space of the whole animal can be calculated from the biological half life of the tracer, the rate of the constant infusion and the concentration of the tracer in plasma water in the steady state for EDTA that ensues when the renal losses of the tracer are balanced by the constant infusion (Moore et al. 1968). The following formula was used:

$$V = (I \times T_{1/2} \times 1.44) / S_p$$

where V = EDTA space of the whole animal, I = intake (=output) of the tracer, $T_{1/2}$ = biological half life of the tracer, S_p = concentration of tracer in plasma water.

Conventional statistical methods and tables (Armitage 1971) were used. Inter-group and intra-group mean values were compared according to Student's t test for independent and dependent observations. When the variance differed considerably the mean values were compared using the F test described by Welch (Welch 1947). The method errors were calculated with analysis of variance. Values are given as mean and the standard deviation.

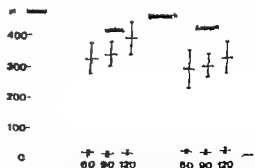
RESULTS

Table 1 gives the number and body weight of the animals in the different experimental groups.

In Table 2 initial and final blood pressure, plasma water volume and EDTA space at the end of the experimental period are presented. The blood pressure values and the plasma water volumes in the groups were the same, while the EDTA space increased with the duration of the infusion. The difference was significant between the 60 min and the 120 min groups.

In Table 3 the error of the method for calculating the volumes of plasma water and EDTA space selected tissues is presented.

In Figs. 1-4 histograms are presented showing the plasma water and EDTA equilibration volume for all the tissues. Compared with the 60 min infusion the plasma water volume tended to decrease in most of the tissues after 90 min EDTA infusion. This decrease was, however, significant only in spleen ($P < 0.05$), the large intestine ($P < 0.05$) and skeletal muscle ($P < 0.05$). The liver, however, increased its plasma water volume ($P < 0.05$).

Fig. 2 Plasma water volume (hatched area) and EDTA space of fundus and antrum of the stomach after EDTA infusion of different duration. Mean \pm S.D. Significant differences are indicated ($P < 0.05$) ($P < 0.01$) ($P < 0.001$).

- MAN H VIER, M & MAGEE M 1953 The metabolism of C^{14} -labeled EDTA in the rat. *J Biol Chem* 204: 1045-1053
- MAN H & TRUJILLO T T 1954 The metabolism of C^{14} -labeled EDTA in human beings. *J Lab Clin Med* 43: 566-571
- NETT E S, PARSONS V & VEALL, N 1967 Measurement of glomerular filtration-rate in man using ^{51}Cr -edetate-acid complex. *Lancet* i: 818-819
- LIČEK, F, BOHNE, F & ZORN H. 1968. Metabolismus und Toxizität therapeutischer Chelatwörter. 4 Mitteilung: Exkretion und metabolischer Abbau von ADTA und DTPA. *Strahlentherapie* 136: 64-68
- WINE, E E, NOWAM, A A, EDISS, C. & STEBE L I 1976 Toxicity, tissue distribution and excretion of $^{46}ScCl_3$ and ^{46}Sc EDTA in mice. *Int J Appl Radiat Isot* 27: 373-377
- DEGAARD-PEDERSEN H J & ENGELL, H C 1972 A comparison of the distribution volumes of radio and C^{14} EDTA in man and nephrectomized dogs. *Scand J Clin Lab Invest* 30: 271-274
- SSON M, JOHNSON L, NYLANDER, G. & JHMAN U. Kinetics of ^{51}Cr EDTA in the rat. Submitted for publication
- MOORE, F D, HARTSUCK J M, ZOLLINGER, R. M J & JOHNSON J E. 1968. Reference models for clinical studies by isotope dilution. *Ann Surg* 168: 671-700
- PRPIC, B 1967 Der Stoffwechsel von ^{45}Ca -EDTA in der Ratte. *Acta Isotop (Padova)* 7: 41-47
- STACY B D & THORBURN G D 1966. Chromium-51 ethylenediaminetetraacetate for estimation of glomerular filtration rate. *Science* 152: 1076-1077
- VIRGILIO R, W HOMER, L D, HERMAN C M, MOSS G S, LOWERY B D. & SCHWARTZ, S L. 1970 Comparison of inulin and chromium-EDTA spaces in the nephrectomized baboon. *J Surg Res* 10: 370-376.
- VOLF V, VLADÄR, M. & SEIDEL, A. 1971 Distribution of labelled Calcium- Yttrium and Chromium-chelates of EDTA in rats. *Arch Int Pharmacodyn Ther* 190: 110-123
- WELCH B. L. 1947 The generalization of "Student" problem when several different population variances are involved. *Biometrics* 34: 28-35

The plasma water volume of the whole animal was unaffected by the length of experimental time and gave consistently normal results in all three groups (de Boer et al 1975).

The results concerning the plasma water volume of the tissues should be regarded with some caution since variations could be attributed in part to a variable blood loss during the process of dissection (Everett et al 1956). The determination of the plasma water volume serves in this study mainly as a control to answer the following question: Is any observed change of the EDTA space due to a change of the plasma water volume and thus possibly an artifact?

The estimation of the method error shows that only a small degree of the total variance in the calculation of plasma water and EDTA equilibration volumes depends on imperfect tissue sampling, sample weighing and isotope counting. However, the differences between tissue samples from the same organ of the same animal contribute a greater part of the total variance while the major portion is due to the biological variation between animals.

Relating the volume of plasma water or EDTA space to the wet weight of the tissue sample causes that any observed change in comparison to the control value will be calculated as a lower figure than the real one assuming no concomitant change in the other fluid volumes. If for example the EDTA space decreases from 250 to 200 $\mu\text{l/g}$ tissue (real reduction) no change occurring in other fluid compartments the calculated reduction of the EDTA space will be from 250 ($=250 \mu\text{l}/1000 \text{ mg tissue}$) to 211 $\mu\text{l/g tissue}$ ($=200 \mu\text{l}/950 \text{ mg tissue}$).

The EDTA space in different tissues showed a uniform tendency to increase with the duration of the EDTA infusion. The increase at 90 min compared to the 60 min values was 14% in the liver and 18% in the large intestine. After 120 min infusion the enlargement of the EDTA space was 34% in the liver, 22% in the gastric fundus, 33% in the large intestine ($P<0.001$), 18% in the small intestine, 30% in the skin ($P<0.01$), 10% in the lung, 13% in the spleen and 17% in the subcutaneous fat ($P<0.05$). The increments of the EDTA space can not be explained by changes in the plasma water volume.

The enlargement of the EDTA space in the liver is of particular interest since this could be due to EDTA excretion in the bile. No attempt was made in these experiments to test this theory. If biliary

excretion of EDTA occurs it is in negligible amounts but has been denied by most authors (Bröchner Mortensen et al 1969, Downes & McDonald 1964, Foreman et al 1953, Gundersen 1967, Prypc 1967, Havlicek et al 1968, Løfberg & Pedersen & Engell 1977).

Both determinations of the total EDTA space of the animal and the EDTA space of different tissues suggest that the tracer penetrates into the extracellular fluid space. Thus when using EDTA as a determinant of changes in the extracellular space it is necessary to minimize and standardize the length of the infusion time in the experimental animals and their controls.

This investigation was supported by a grant from the Swedish Medical Research Council (project No 17X-020-13C).

REFERENCES

- AHRENS F A & ARONSON A L 1971 A comparative study of the toxic effects of calcium and chelates of ethylenediaminetetraacetate in the rat. *Toxicol Appl Pharmacol* 19, 10-3.
- ALTMAN P L & DITTMER D S 1961 Blood and other body fluids. Federation of American Societies for Experimental Biology, Washington, D.C.
- ARMITAGE P 1971 Statistical methods in medical research. Blackwell Scientific Publications, Oxford.
- BERNARD J P & BIRON A 1971 Mesure de la fraction extracellulaire rapidement échangeable (LECCE) par EDTA ^{51}Cr . Etude chez le chien anesthésié. *Chimie biologique* 1, 291-296.
- DEBOER J, ARCHIBALD J & DOWNES H G P 1967 An introduction to experimental surgery. Elsevier Medical, Amsterdam.
- BOHNE F, HARMUTH HOENE A, KURTZ T, K & HAVLICEK F 1968 Metabolismus und Toxizität therapeutischer Chelatbildner. 9. Mitteilung: physiologische Verdünnungsraum des EDTA. *DTPA Strahlentherapie* 136, 609-616.
- BRADING A F & JONES A W 1969 Distribution and kinetics of CoEDTA in smooth muscle and liver: an extracellular marker. *J Physiol (Lond)* 208, 401.
- BRÖCHNER MORTENSEN J, GIESE J & JENSEN S 1969 Renal and plasma clearance versus plasma clearance of ^{51}Cr EDTA. *Scand J Clin Invest* 23, 301-305.
- DOWNES A M & McDONALD I W 1964 Chromium 51 complex of ethylenediamine tetraacetic acid as a soluble rumen marker. *Br J Nutr* 18, 141-144.
- EVERETT N B, SIMMONS H & LASHLEY R F 1946 Distribution of blood (P^{32}) and plasma volumes of rats determined by liquid nitrogen freezing. *Circ Res* 4, 419-424.

traction of the rat portal vein in hypertonic Isotonic medium mechanical properties and cts of Mg^{2+}

HELLSTRAND and ANDERS ARNER

ment of Physiology and Biophysics, University of Lund, Sweden

HELLSTRAND P & ARNER A. Contraction of the rat portal vein in hypertonic and isotonic medium: mechanical properties and effect of Mg^{2+} . *Acta Physiol Scand* 1980; 110: 49-67. Received 4 Dec 1979. ISSN 0001-6772. Department of Physiology and Biophysics, University of Lund, Sweden.

The contracture elicited in the rat portal vein by hyperosmotic solutions (HC) has been investigated with respect to its Mg^{2+} -dependence and its mechanical properties. Comparison was made with K^+ -induced contractures (KC) of similar force. In contrast to its relative resistance to Ca^{2+} -depletion, HC is entirely abolished after depletion of both Ca^{2+} and Mg^{2+} ions. After readdition of Mg^{2+} alone, HC can be partially restored again. Isotonic quick release experiments were performed on arteries in HC, KC and after passive stretch (PS) to a similar total tension and the length responses following the releases were analyzed. The immediate elastic response showed that stiffness was markedly higher in HC than in KC, whereas in PS it was much lower. After the elastic response, a quickly decaying exponential component could be distinguished from the later more steady length change. The time constant of this transient response was 40-50 ms (KC) or 25-30 ms (HC). On release to a minimal afterload, the amplitude of the transient response was about 1% (KC) or 0.5% (HC) of the initial muscle length, decreasing with increasing afterload. A corresponding transient lengthening response was seen after a step to a force greater than the isometric tension. Transient length responses in PS were much smaller than in KC or HC (following force steps producing similar elastic recoil). The late shortening in HC and KC, interpreted as the viscous response of the contractile system, could be fitted to Hill's eq. V_{max} in KC ($[Ca^{2+}] = 0.5$ mM) was 25 ± 0.62 and in HC 0.05 ± 0.01 (SE, $6 \mu\text{length/s}$). The results of this investigation support the conclusion that HC originates in the contractile system of the muscle. The quantitative differences in mechanical properties are discussed against possible alterations in HC of cross-bridge behaviour and/or filament structure.

Key and Smooth muscle hyperosmolality Mg^{2+} contraction, mechanical transients, elastic recoil force-velocity relation

Unbalanced tension increase of the rat portal vein in some hyperosmotic solutions has been described and was shown to be largely independent of external Ca^{2+} supply (Andersson et al 1974). However, the response was found to be greatly inhibited at low temperature and during metabolic action, suggesting that it is due to activation of contractile system of the muscle. The mechanical behaviour of the portal vein in hyperosmotic solution indicated a greater stiffness and a lower shortening speed than in a K^+ -induced contracture of similar force. In the present study the ionic dependence and mechanical properties have been

further characterized, including an analysis of the transient length changes occurring in the first few milliseconds after a force step (Johansson Hellstrand & Uvelius 1978; Hellstrand & Johansson 1979). The results indicate a marked Mg^{2+} -dependence of the hyperosmolality contracture, providing further evidence that it is caused by activation of the contractile proteins of the smooth muscle. There are however large quantitative differences in mechanical properties between the hyperosmolality contracture and the K^+ -induced contracture, suggesting that the contractile system may be in quite different states.

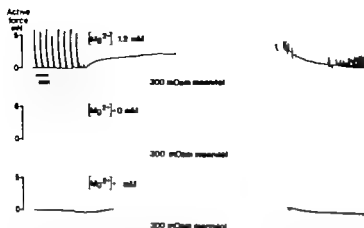


Fig. 1 Upper panel: response of portal vein to hyperosmolar solution (300 mOsm mannitol) added to normal medium. Middle panel: response after 60 min exposure to Ca^{2+} -free, Mg^{2+} -free solution containing 1 mM EDTA. Lower panel: response 15 min after addition of 5 mM Mg^{2+} .

der is effectively deplete intracellular Mg^{2+} . It is necessary to expose the muscle to a solution free of both Mg^{2+} ions and Ca^{2+} ions (Anderson & Uvelius 1977). In Fig. 1 the upper panel shows control response of a portal vein in a solution of normal ionic composition when osmolality is increased by the addition of 300 mOsm/kg mannitol. The hyperosmotic solution was then replaced by one of normal osmolality lacking added Ca^{2+} and Mg^{2+} and with 1 mM EDTA present to sequester trace amounts of these ions. 60 min later the osmolality response was completely absent (middle panel). On exposure to the hyperosmotic solution 15 min after the addition of 5 mM Mg^{2+} a weak contracture appeared (lower panel) (but no Ca^{2+} ions were added). Addition of 1 mM Ca^{2+} alone with no Mg^{2+} present was not sufficient to restore the HC after divalent cation depletion. After return to normal medium the muscle resumed spontaneous activity although this was of low frequency and low amplitude. The Mg^{2+} sensitivity of HC was consistently seen in 11 experiments.

Isometric response. Isometric experiments were performed to characterize the dynamic behaviour of the portal vein in HC as compared to KC and to a condition of relaxed passive tension, produced by stretch. Some data on the force-velocity relation and active properties were reported by Anderson (1974), but the present experiments have been performed with much improved time resolution, allowing

steady afterload after a force step to be established in a few ms (Sjölin et al. 1978). Fig. 2 shows superimposed representative length responses in KC, HC and PS. The relative force steps P/P_0 were

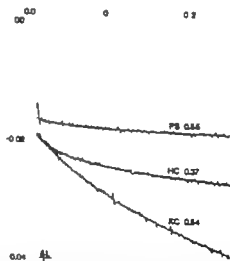


Fig. 2 Superimposed length responses to force steps in portal vein. Records were digitized at 1 kHz and displayed together with double-exponential fit (broken lines). Downward deflection marks shortening. Relative force step given by number beside each tracing. Upper tracing: passive stretched muscle (initial tension 3.1 mN, length 6.3 mm, weight 2 mg), middle tracing: hyperosmolality contracture (initial tension 3.6 mN, length 4.4 mm), lower tracing: K⁺-induced contracture (initial tension 4.7 mN, length 4.4 mm). Length responses normalized to the short or length.

In a separate paper (Arner & Hellstrand 1980) the metabolic flux rates associated with responses to hyperosmolar or K^+ high solutions are reported.

METHODS

Portal veins were dissected from male Sprague-Dawley rats (200–300 g) and cleaned of fat and adventitial connective tissue. They were mounted and left to accommodate for at least 45 min in a solution ("normal tris") of the following composition in mM: NaCl 170, KCl 6.0, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 11.5, Na_2Ca -versenate 0.026 and tris (hydroxymethyl)-aminomethane (Trizma Base, Sigma Chemical Co.) 23 at pH 7.4 bubbled with 100% O_2 . Nominally Ca^{2+} -free tris solution was prepared as normal tris except that Ca^{2+} was omitted. Temperature was kept at 37°C throughout the experiments. Contraction was elicited either by a solution where 100 mM NaCl of the normal tris had been replaced by KCl (i.e. high tris) or by a solution made hyperosmotic by the addition of mannitol to the normal tris. To make these two kinds of contraction similar in magnitude it was necessary to use a relatively low $[Ca^{2+}]$ (0.5 mM) in the K^+ high tris. Some experiments were performed in Mg^{2+} and/or Ca^{2+} -free solutions containing EDTA (1 mM) to chelate the divalent cations. Osmolality of the solutions used was routinely checked on an Advanced Instruments Inc. freezing point osmometer (31-LAS) and amounted to about 290 mosm/kg H_2O in the normal medium and 590 mosm/kg H_2O in the hypertonic solution. Two kinds of mechanical experiments were performed: (i) isometric recording in a 50 ml organ bath using Grass FT03 force transducers; (ii) isotonic quick release experiments using the apparatus described by Sjölin, Hellstrand & Clementz (1978). In the latter experiments the force and length records were stored on magnetic tape and subsequently digitized at a sampling frequency of 1 kHz for computer analysis (Hellstrand & Johansson 1979). The program performs a fit of the length record following the initial elastic recoil to a model consisting of a sum of two decaying exponential functions:

$$L(t) = L + A(1 - e^{-t/\tau}) + A_s(1 - e^{-t/\tau_s}) \quad (1)$$

Here L is the muscle length at $t=0$ after the end of the elastic recoil, A and τ are the amplitude and time constants respectively of a fast ($\tau=1$) and a slow ($\tau=2$) exponential phase. For shortening of the muscle $A_s < 0$ and for lengthening $A_s > 0$. In such an analysis performed on rabbit bladder smooth muscle (Hellstrand & Johansson 1979) the fast and slow phases could be distinguished by different dependence on the level of activation of the muscle, whereas the temperature dependence was found to be similar.

The shortening velocity at 100 ms after release was obtained by differentiation from the double exponential fit. This was used for construction of force-velocity curve by a computer fit to the Hill equation:

$$V = (P - P_0)/(P + a) \quad (2)$$

The series elastic (SE) recoil were determined as the intersection of the fitted double-exponential or the length record during the elastic response were fitted to a logarithmic curve:

$$\frac{\Delta L}{L} = \frac{1}{k} \times \ln \frac{P-B}{A}$$

where A , B and k are constants and L is the muscle length. In construction of SE force-extension records with $P > P_0$ as well as $P < P_0$ are both force-velocity curves computed according to eq. 2 with $P < P_0$ were used.

In the quick release experiments the muscle was first in normal tris at a low preload (< 0.75 mN). Experiments with K^+ high solutions were then performed in the hypertonic solution in 3 of the experiments, K^+ high in the 3 others. K^+ -contractures (KC) were induced by repeatedly exposing the muscle to K^+ solution for 1 min followed by normal tris (0.5 mM Ca^{2+} min). When reproducible contractions had been achieved quick releases to varying afterloads ($P < P_0$) were performed in a random order. In some of the experiments stretches ($P > P_0$) in order of rising afterload were performed after all the releases had been made. In the hypertonic contractures (HC) all releases and stretches were performed while the muscle remained contracted. No loss of contractility was seen during this procedure. Between trials the muscle was allowed to recover for at least 1 min in normal tris (2.5 mM Ca^{2+}).

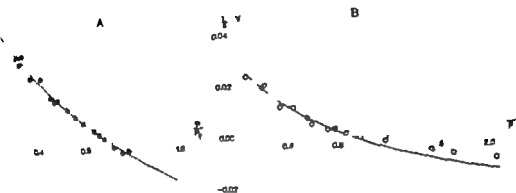
After the HC and KC responses had been determined the muscle was relaxed in nominally Ca^{2+} -free normal osmolality. It was then stretched until its tension equalled the total force reached in the last responses, and kept at this new length. A series of stretches and stretches of the relaxed muscle was then recorded in a similar way as before.

At the end of the experiment the muscle was returned to its original length, which was determined by microscopy on an ocular scale. It was then removed, blotted, weighed on a Cahn electrobalance. Cross-sectional area was computed from the length and wet weight and uniform thickness and a density of 1.05 mg/mm³.

RESULTS

1. Mg^{2+} -dependence of the hyperosmolarity contracture (HC)

The contracture elicited in the portal vein by highly hyperosmotic solutions persists even if extracellular $[Ca^{2+}]$ is reduced drastically below the limit for contraction evoked by other stimuli (Andersson *et al.* 1974). We observed however that the hyperosmolarity contracture (HC) is critically dependent on Mg^{2+} ions and in that respect resembles other types of active contraction in the smooth muscle. Removing Mg^{2+} from the external solution does not affect either HC or K^+ -induced contractures.



Force-velocity relations in K-contraction (A) and in hyperosmolarity contraction (B). Same experiment as in Fig. 3. Forces measured 100 ms after release. Note different scales on velocity axis.

KC: open circles and broken line from HC, crosses and full line from PS. Parameters of the curve are given in the legend. It is evident that the muscle is stiffer in HC than in either KC or PS. A comparison of mechanical parameters in HC and control muscles is shown in Table 1. It is seen that the dimensionless constant k from eq. (3), which characterizes the stiffness of an exponential spring, is significantly greater in HC despite similar levels of developed stress.

Force-velocity relation

The shortening record in the first 50–100 ms following release is seen from Fig. 2 to be characterized, in the cases of KC and HC, by a rapidly declining velocity as shortening proceeds. Such behaviour is seen also in PS, but the muscle here behaves essentially as passive elastic (Fig. 2). The phenomenon of rapidly diminishing shortening velocity may be generally occurring in all three muscle types but can be analyzed only in experiments made with sufficient time resolution (see below, Hellstrand & Ulfhøj 1978; Hellstrand & Johansson 1979; Mulvany 1979). To specify the force-velocity relation it is necessary to fix a point in time after the release at which the velocity is to be measured. In this and earlier work we have measured velocity at 100 ms after release, since the most rapid shortening has essentially then and the velocity may be consid-

ered characteristic of the "steady state" behaviour of the contractile system (Hellstrand & Johansson 1979).

Representative force-velocity records at 100 ms in KC and HC are shown in Fig. 4 A and B respectively. It is seen that shortening, as well as lengthen-

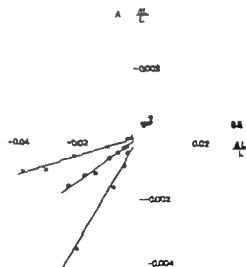


Fig. 5. Amplitude A_4 of exponential monotonic transient (cf eq. (11)), plotted against magnitude of immediate elastic recoil (SE). Same experiment as in Fig. 3. Open circles, hyperosmolarity contraction; filled circles, K-contraction; crosses, passive stretched muscle.

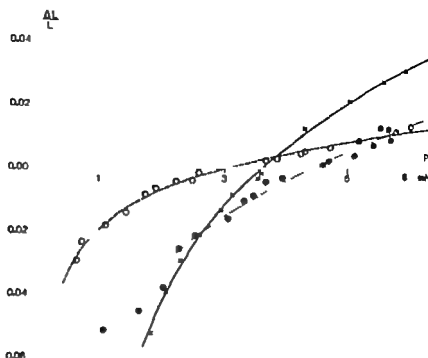


Fig. 3 Force-extension relations of initial elastic response. Logarithmic fit to eq. (1) shown. Data from one muscle. Open circles, broken line: hyperosmolarity contracture (muscle length 6.8 mm, weight 1.95 mg; parameters of fit $k=69.3$, $A=0.84$ mN, $B=0.28$ mN). Filled circles, dotted line: k -contracture (length 6.8 mm, $k=50$, $A=4.50$ mN, $B=-0.5$ mN). Crosses, full line: passive muscle (length 9 mm, $k=29$, $A=1.84$ mN, $B=1.58$ mN). In the passive muscle, length responses were normalized to the shorter muscle length of the other two plots, to allow comparison of absolute magnitudes.

chosen to give elastic recoils of similar magnitudes and are shown by the number beside each tracing. The output from the length transducer was digitized at 1 kHz and then displayed graphically. Superimposed on the length tracings are the double-exponential fits calculated according to eq. (1) (see Methods). In HC and KC the preload on the muscle was kept low (0.6 mN) to minimize influence of parallel elasticity, whereas in PS the passive muscle in Ca^{2+} -free solution had been extended by 43% of its original length to produce a force of 3.1

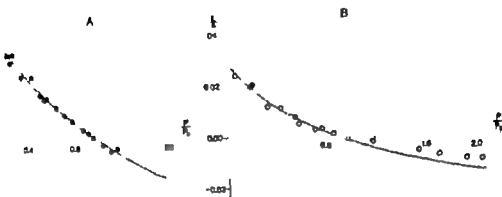
mN. It is seen that although the elastic recoil is about the same magnitude, the force step produced by this recoil is the greatest in HC, smaller in KC, substantially smaller in PS. Thus, the stiffness is greatest in the HC response. Clearly, due to the rather small active tension developed by KC, the fact that $[Ca^{2+}]$ was kept as low as 0.9 μ M in KC to produce similar active tension, parallel elasticity cannot strictly be neglected in these experiments. However, no attempt has been made to correct for this, since such a correction is likely to be very unreliable and depends critically on the assumptions made as to the geometrical arrangement of the functional mechanical elements in the muscle. The comparisons made here must therefore necessarily be regarded as qualitative. Emphasis is placed on the comparison of HC and KC, since they are at the same total length of the muscle and presumably therefore at the same level of tension of the passive elements.

In Fig. 3 are shown logarithmic fits according to eq. (3) made to the elastic responses to force steps of different magnitudes. All data are obtained from one muscle. Filled circles and dotted line show

Table 1. Mechanical parameters of portal veins in KC and HC

Mean values \pm SE of 6 expts. P_0 , a and b defined by eq. (2), $V_{max}=bP_0/a$. The dimensionless stiffness parameter k defined by eq. (3). $P<0.01$, $P<0.001$ by paired Student's t -test.

		k -contr	Hyperosm
P	mN/mm ²	9.9 ± 1.3	9.8 ± 0.8
k	—	34 ± 3	55 ± 5
V_{max}	length/s	0.5 ± 0.0	0.05 ± 0.01
a/P_0	—	3.0 ± 0.6	7 ± 1.4
b	length/s	0.68 ± 0.1	0.07 ± 0.04



Force-velocity relations in K-contracture (A) and in hyperosmolarity contracture (B). Same exp't as in Fig. 3 measured 100 ms after release. Note different scales on velocity axis.

C: open circles and broken line from HC, crosses and full line from PS. Parameters of the fit are given in the legend. It is evident that the fit is stiffer in HC than in either KC or PS. A ratio of mechanical parameters in HC and in 6 muscles is shown in Table 1. It is seen that the dimensionless constant k from eq. (3) characterizes the stiffness of an exponential, and is significantly greater in HC, despite similar values of developed stress.

Force-velocity relation

The shortening record in the first 30–100 ms following release shows a characteristic response is seen from Fig. 2 to be characterized in the cases of KC and HC by a rapidly declining velocity as shortening proceeds. A similar behavior is seen also in PS, but the latter behaves essentially as a passive elastic.

The phenomenon of rapidly diminishing shortening velocity may be generally occurring in skeletal muscle, but can be analyzed only in experiments made with sufficient time resolution (Larsson, Hellstrand & Uvelius 1978, Hellstrand & Larsson 1979, Mulvany 1979). To specify the force-velocity relation it is necessary to fix a point in time after the release at which the velocity is to be measured. In this and earlier work we have measured velocity at 100 ms after release, since the velocity-independent rapid shortening has essentially ended by then and the velocity may be consid-

ered characteristic of the steady state behaviour of the contractile system (Hellstrand & Johansson 1979).

Representative force-velocity records at 100 ms in KC and HC are shown in Fig. 4 A and B respectively. It is seen that shortening, as well as lengthen-

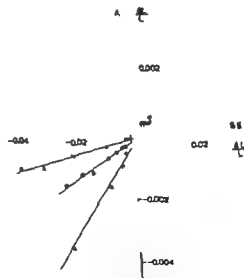


Fig. 5. Amplitude A_2 of exponential isotonic transient [cf. eq. (1)], plotted against magnitude of immediate elastic recoil (SE). Same exp't as in Fig. 3. Open circles: hyperosmolarity contracture. Filled circles: K-contracture. Crosses: passive stretched muscle.

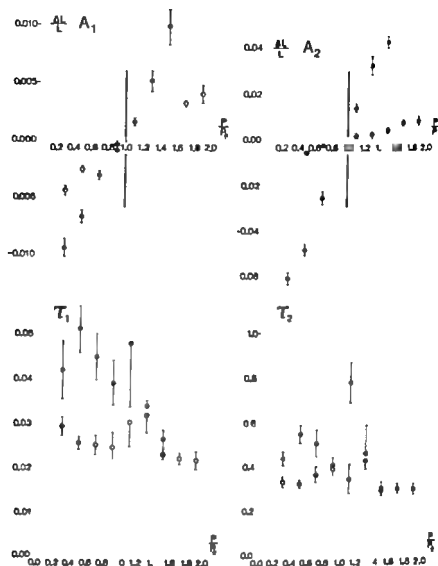


Fig. 6 Amplitudes (A_1 , A_2) and time constants (T_1 , T_2) of exponential components in fits to length records according to eq. (1). Filled circles, K^+ -contracture. Open circles, hyperosmolality contracture. Data from 6 expts. grouped according to P/P_0 into classes with a width of 0.2. Mean values \pm S.E. shown. Each point represents 3–16 values. Broken lines emphasize transition between shortening and lengthening responses.

ing velocities are considerably lower in HC (note the difference in scale). The hyperbolae are fitted to the points with $P < P_0$ according to Hill's eq. (?) Force-velocity points for $P > P_0$ at or above the continuation of the hyperbola were seen in 5 expts. out of 6 in HC, whereas in KC they consistently fell below the hyperbola (cf. Johansson et al. 1978).

The constants characterizing the force-velocity relations (eq. ?) a/P_0 , b and maximal shortening velocity $V_{max} = b \times P_0/a$ found in the 6 expts. are shown in Table 1 (mean values \pm S.E.). Both V_{max} and b are considerably lower in HC than the difference being highly significant. Values for a/P_0 are not significantly different. They are rather high and

spread considerably (cf. Hellstrand 1979). This may be a consequence of the relatively small development as compared to preload in the present experiments.

4. Transient isometric responses

The time-dependent response to a force (isotonic transient) with rapidly damped shortening (or lengthening) velocity was analysed in a manner analogous to that used on rabbit bladder muscle by Hellstrand & Johansson (1979). The approach is to treat the length record as the sum of two exponential components (eq. (1)), each characterized by its amplitude and time constant (Methods). Isotonic transients were seen in the

as on the portal vein both in KC and in HC as also a phenomenon of similar time scale (constant 20-50 ms) in the releases made in PS responses, particularly at low afterloads, however not be fitted with certainty to the exponential model due to prominent inertial terms.

Fig. 5 (data from 1 exp't) shows that relative to the recoil (SE), the amplitude (A_1) of the isotonic transient is largest in KC smaller in HC and smaller in PS.

In Fig. 6 are plotted the amplitudes A_1 and A_2 and the constants τ for the HC and KC series. Data from 6 exp'ts were grouped into two.

According to PYP, PS responses are not fitted; no fits could not be obtained with certainty and since ambiguities of interpretation may arise due to the different muscle lengths (note that not a problem in Fig. 5 where both axes are fitted to muscle length). The amplitudes A_1 of the exponential components is smaller in absolute magnitude in HC than in KC both for shortening and lengthening responses. The time constant τ for shortening responses, smaller in HC than in KC. The slow exponential component had considerably smaller amplitude (A_2) in HC than in KC but a time constant (τ). This corresponds to the τ velocity in KC as already seen.

In the stretch responses (to the right of the diagram) the amplitudes A_1 and A_2 and the time constants show considerable scatter in small force steps. They generally seem to be with greater force step, but the tendency is not clear and cannot be judged with certainty. Note here a difference of an order of magnitude between τ and τ_1 implying that the separation into processes is quite reliable.

DISCUSSION

Hyperosmolarity contracture (HC) was found to be dependent on extracellular Mg^{2+} ions to the same extent as the spontaneous activity of the contractile element in the portal vein by K^+ -high medium (Sapardov & Uchida 1977). This is in contrast to the relative resistance to Ca^{2+} -depletion (Anderson et al. 1974). Evidence has been presented that HC is an active response of the muscle rather than a consequence of passive cell shrinkage (Anderson et al. 1974; McGrath & Shepherd 1976).

The Mg^{2+} -dependence of HC suggests that Mg^{2+} -dependent (actomyosin) ATPase is involved in the response.

The low sensitivity of HC to Ca^{2+} depletion might indicate either that in the hypertonic solution Ca^{2+} ions are released from intracellular stores or that the contractile system becomes sensitive to very low Ca^{2+} levels or altogether independent of Ca^{2+} regulation. It is interesting that in chemically skinned smooth muscle cells a moderate elevation of the Mg^{2+} concentration of the medium is able to elicit contraction even in the absence of Ca^{2+} ions (Saito & Nonomura 1978). Ca^{2+} -independent ATPase activity occurs in regulated actomyosin systems from skeletal muscle *in vitro* when the level of ATP is low (Weber & Murray 1973). This phenomenon is attributed to the presence of some attached rigor complexes removing the inhibition of the active sites normally effected by the tropomyosin molecule in the absence of Ca^{2+} ions. No evidence for such a mechanism in smooth muscle has however been presented, to our knowledge.

Although activation by Ca^{2+} probably accounts at least in part for HC in the portal vein there remains the possibility that Mg^{2+} -dependent ATPase activity is involved in the hyperosmotic response independent of Ca^{2+} regulation.

The mechanical experiments on the portal vein in hypertonic solution indicate an increased stiffness, both with respect to the relaxed state in normal solution and to the K^+ -induced contracture (KC Fig. 3). In addition, the shortening velocity is greatly reduced compared to that in KC (Fig. 4 and Table 1). Qualitatively these effects were noted earlier (Andersson et al. 1974). The isotonic transient responses seen in the portal vein and other smooth muscles (Johansson et al. 1978; Mulvany 1979) have tentatively been interpreted in terms of a cross-bridge mechanism of contraction (Helfstrand & Johansson 1979). In the present study it is shown that both the amplitude (A_1) and the time constant (τ) of the exponentially decaying isotonic transient length response are reduced in HC as compared to a KC of similar force (Fig. 5 and 6).

Phenomenologically the transient mechanical responses in smooth muscle may be thought to result from an undamped and a damped elasticity connected in series in a Voigt element (Mulvany 1979). In terms of this model the present results would mean that both springs have become stiffer in the hypertonic solution although not proportionally.

so as shown by Fig 5. In a linear Voigt model the time constant τ_1 would be determined by the quotient viscosity/stiffness of the damped element. If a linear approximation is applicable the results in Fig 6 (τ_1) would mean that in HC the quotient is reduced. The active shortening subsequent to the transient responses after a force step is not explained by the viscoelastic model but requires the presence of an independent force or shortening generator (cf Mulvany 1979). The greatly reduced shortening velocity in HC compared to KC (Fig 4 A and B) will thus mean that the generator has profoundly altered its properties in the hyperosmotic environment.

Differences in shortening velocity and metabolic demand of contraction between different kinds of muscle have been considered against differences in the length of the contractile units (sarcomere analogues) of the muscles (Rüegg 1971). Thus in muscles with longer contractile units shortening velocity would be smaller at equal rate of turnover of cross bridges. One would also predict that length transients representing relaxation phenomena within the individual sarcomere analogues would have a smaller overall amplitude in a muscle where these units are longer. The thick filaments in taenia coli smooth muscle were suggested by Lowy & Small (1979) to exist in the form of long ribbon-like structures. However Somlyo, Somlyo & Devine (1971) showed in expts on the rabbit portal vein that ribbons were produced consistently when the muscles were stretched and/or treated by hyperosmotic solutions similar to those used by us where as round filaments were seen at normal tonicity. In subsequent expts the length of these round filaments were determined to be about $2.2 \mu\text{m}$ (Ashton, Somlyo & Somlyo 1975) thus somewhat longer than the thick filaments of skeletal muscle. It is an interesting possibility that the morphological characteristics of portal veins in hyperosmotic solution may be related to the mechanical properties reported here. Thus if ribbons are present it is conceivable that the contractile units of the muscle functionally behave as if their length had been considerably increased. It is entirely plausible however that the hyperosmotic solution will cause biochemical alterations in the muscle affecting the rate and extent of cross bridge turnover. Expts relating to the metabolic demands of contraction in KC and HC are reported in the concomitant paper (Arner & Hellstrand 1980). They indicate that in hyperosmotic

medium the cross-bridge cycle operates essentially normal way but that the number of cross bridges may be reduced. This suggests that the increased stiffness and loss in hypertonicity are caused by some factor reducing internal resistance to length change.

We would like to thank Monica Lundblom, Heidenholm and Ina Nordström for technical assistance. The investigation was supported by grants from Swedish Medical Research Council 1015-80, Medical Faculty, University of Lund and ABT Göteborg.

REFERENCES

- ANDERSSON C, HELLSTRAND P, JOHANSSON B & RINGBERG A 1974 Contraction in smooth muscle induced by hypertonicity: dependence and mechanical characteristics. *Acta Physiol Scand* 90: 451-461.
- ARNER A & HELLSTRAND P 1980 Contraction in the rat portal vein in hypertonic and normotonic solutions: rates of metabolism. *Acta Physiol Scand* 110: 114-118.
- ASHTON F T, SOMLYO A Y & SOMLYO V P 1975 The contractile apparatus of vascular smooth muscle: intermediate high voltage electron microscopy. *J Molecular Biol* 98: 17-49.
- HELLSTRAND P 1979 Mechanical and electrical properties related to contraction in smooth muscle. *Acta Physiol Scand Suppl* 464: 1-14.
- HELLSTRAND P & JOHANSSON B 1979 A study of the length response to a force step in smooth muscle from rabbit urinary bladder. *Acta Physiol Scand* 106: 1-13.
- JOHANSSON B, HELLSTRAND P & UUTERLUND 1978 Responses of smooth muscle to length change studied at high time resolution. *Br J Physiol* 15: 63-87.
- LOWY J & SMALL J V 1970 The organization of myosin and actin in vertebrate smooth muscle. *J Cell Biol* 7: 46-51.
- MCCRATH M A & SHEPHERD J T 1976 The ionic molar effect on nerves and smooth muscle. *Am J Physiol* 231: 141-147.
- MULVANY M J 1979 The undamped and damped elastic components of a vascular smooth muscle. *Biophys J* 26: 401-414.
- RÜEGG J C 1971 Smooth muscle tone. *Physiol Rev* 51: 201-48.
- SAIDA K & NONOMIURA Y 1978 Characteristics of Ca^{2+} and Mg^{2+} induced tension development in mechanically skinned smooth muscle fibers. *J Physiol* 273: 1-14.
- SIGURDSSON S B & UUTERLUND B 1977 The effect of variation in extracellular magnesium concentration on electrical and mechanical activity in rat portal vein. *Acta Physiol Scand* 99: 3.

22. LIN, L., HELLSTRAND H. & CLEMENTZ, B. 1978. An apparatus for mechanical experiments on isolated smooth muscle. *Acta Physiol Scand* 102: 2A-33A.
- SOMLYO A. P., SOMLYO A. V. DEVINE, C. E. & RICE, R. V. 1971. Aggregation of thick filaments into ribbons in mammalian smooth muscle. *Nature New Biol* 231: 243-246.

as shown by Fig 5. In a linear Voigt model the time constant τ would be determined by the quotient viscosity/stiffness of the damped element. If a linear approximation is applicable the results in Fig. 6 (τ) would mean that in HC this quotient is reduced. The "active shortening subsequent to the transient responses after a force step is not explained by the viscoelastic model but requires the presence of an independent force or shortening generator (cf. Mulvany 1979). The greatly reduced shortening velocity in HC compared to KC (Fig. 4 A and B) will thus mean that the generator has profoundly altered its properties in the hyperosmotic environment.

Differences in shortening velocity and metabolic demand of contraction between different kinds of muscle have been considered against differences in the length of the "contractile units" (sarcomere analogues) of the muscles (Rüegg 1971). Thus in muscles with longer contractile units shortening velocity would be smaller at equal rate of turnover of cross bridges. One would also predict that length transients representing relaxation phenomena within the individual sarcomere analogues would have a smaller overall amplitude in a muscle where these units are longer. The thick filaments in taenia coli smooth muscle were suggested by Lowy & Small (1979) to exist in the form of long ribbon-like structures. However Somlyo, Somlyo & Devine (1971) showed in expts on the rabbit portal vein that ribbons were produced consistently when the muscles were stretched and/or treated by hyperosmotic solutions similar to those used by us where as round filaments were seen at normal tonicity. In subsequent expts the length of these round filaments were determined to be about $2.2 \mu\text{m}$ (Ashton, Somlyo & Somlyo 1975) thus somewhat longer than the thick filaments of skeletal muscle. It is an interesting possibility that the morphological characteristics of portal veins in hyperosmotic solution may be related to the mechanical properties reported here. Thus if ribbons are present it is conceivable that the contractile units of the muscle functionally behave as if their length had been considerably increased. It is entirely plausible however that the hyperosmotic solution will cause biochemical alterations in the muscle affecting the rate and extent of cross-bridge turnover. Expts relating to the metabolic demands of contraction in KC and HC are reported in the concomitant paper (Arner & Hellstrand 1980). They indicate that in hyperosmot-

ic medium the cross-bridge cycle operates essentially normal way but that the number of active cross bridges may be reduced. This suggests that the increased stiffness and lower v in hypertonicity are caused by some factor(s) reducing internal resistance to length change.

We would like to thank Monica Lundahl & Heidenholm and Ina Nordström for technical assistance. The investigation was supported by grants from Swedish Medical Research Council (64X-6034), Medical Faculty, University of Lund and AB H. Göteborg.

REFERENCES

- ANDERSSON C, HELLSTRAND P, JOHANSSON B & RINGBERG A 1974. Contraction in vascular smooth muscle induced by hypertonicity: dependence and mechanical characteristics. *Acta Physiol Scand* 90: 451-461.
- ARNER A & HELLSTRAND P 1980. Contraction of the rat portal vein in hypertonic and isotonic media: rates of metabolism. *Acta Physiol Scand* 110: 64-74.
- ASHTON F T, SOMLYO A V & SOMLYO A 1975. The contractile apparatus of vascular smooth muscle: intermediate high voltage stereo electron microscopy. *J. Molecular Biol.* 98: 17-29.
- HELLSTRAND P 1979. Mechanical and social properties related to contraction in smooth muscle. *Acta Physiol Scand Suppl.* 464: 1-54.
- HELLSTRAND P & JOHANSSON B 1979. Analysis of the length response to a force step in smooth muscle from rabbit urinary bladder. *Acta Physiol Scand* 106: 221-238.
- JOHANSSON B, HELLSTRAND P & UVELIUS U 1978. Responses of smooth muscle to quick length change studied at high time resolution. *Blood Vessels* 15: 65-82.
- LOWY J & SMALL J V 1970. The organization of myosin and actin in vertebrate smooth muscle. *Nature (Lond.)* 227: 46-51.
- MCGRATH M A & SHEPHERD J T 1976. Hypertonicity effect on nerves and smooth muscle: cutaneous vein. *Am J Physiol* 31: 141-147.
- MULVANY M J 1979. The undamped and damped series elastic component of vascular smooth muscle. *Biophys J* 26: 401-414.
- RUEGG J C 1971. Smooth muscle tone. *Physiol Rev* 51: 201-48.
- SAIDA H & NONOMURA Y 1978. Characterization of Ca^{2+} and Mg^{2+} induced tension development in chemically skinned smooth muscle fibers. *J. Physiol* 277: 1-14.
- SIGURDSSON S B & UVELIUS U 1977. The effect of variations in extracellular magnesium concentration on electrical and mechanical activity in rat portal vein. *Acta Physiol Scand* 99: 368-376.

Contraction of the rat portal vein in hypertonic and isotonic medium: rates of metabolism

ARS ARNER and PER HELLSTRAND

Department of Physiology and Biophysics, University of Lund, Sweden

ARNER, A. & HELLSTRAND, P. Contraction of the rat portal vein in hypertonic and isotonic medium: rates of metabolism. *Acta Physiol Scand* 1980, 110: 69-75. Received 4 Dec 1979. ISSN 0003-6772. Department of Physiology and Biophysics, University of Lund, Sweden.

The contraction induced by hyperosmolality (HC) in the rat portal vein has been investigated with respect to its metabolic properties. Oxygen consumption (\dot{V}_{O_2}) and lactate production (\dot{V}_{LA}) are measured. Comparison was made with K⁺-induced contractions (KC). Variation in active force was produced for both kind of contractions by alteration of muscle length. Measurements were also made in normal isotonic solution, where the muscle is spontaneously active, and in Ca^{2+} -free medium solution. At short muscle lengths, where no active tension was produced, \dot{V}_{O_2} was lower in HC than in KC. In contrast, \dot{V}_{LA} was greatly enhanced in HC so that calculated ATP turnover (\dot{V}_{ATP}) was about the same in both kind of solution. Comparison of metabolic rates at long and short muscle length and correlation to force development allowed calculation of tension-dependent metabolism. In normal solution (spontaneous activity) tension-dependent metabolic rate was much greater than in either kind of contracture. No difference in tension-dependent \dot{V}_{O_2} , \dot{V}_{LA} or \dot{V}_{ATP} could be detected between HC and KC. In a Ca^{2+} -free solution, force development and metabolic rates in HC did not differ from those in Ca^{2+} -contracting solution. Glucose caused abolished lactate formation in HC. The temporal variations of \dot{V}_{O_2} and active force during 30 min of HC and KC are followed. In HC force development increased with time whereas in KC it decreased. Tension-independent \dot{V}_{O_2} declined with time in HC but stayed constant in KC. On the other hand tension-dependent \dot{V}_{O_2} was unchanged in HC but declined in KC. The results of this study indicate that despite alterations in overall cell metabolism caused by the hypertonic solution, the energetic aspect of cross-bridge operation may be similar in HC and KC, despite pronounced differences in dynamic mechanical properties.

Key words: Smooth muscle; hyperosmolality; contraction; metabolic rate; oxygen; lactate

It is well known that hypertonic media influence the contractile and metabolic properties of muscle. In skeletal muscle an increased stiffness of the elastic component is seen (Jewell & Whipple 1958) as well as a reduced speed of active shortening (Sjogstrand 1958).

In addition, contracture appears in the nonstimulated muscle on exposure to hypertonic medium (Jarnstrom & Voith 1971). In smooth muscle of the rat portal vein similar mechanical effects were described by Anderson et al. (1974) and have been further characterized in the concomitant paper (Hellstrand & Arner 1980). Metabolic responses in hypertonic media have been investigated in skeletal muscle by measurement of oxygen con-

sumption and lactate formation (Muller 1962, Clemons 1968) as well as of heat production (Yamada 1970). A consistent finding in these investigations was increased metabolic rate in hypertonic media. However, no correlation of metabolic rate with tension development under these conditions has been made. A general relationship between metabolic rate and shortening velocity of muscles has been proposed (Räberg 1971). A greater rate of ATP turnover would thus correspond to a greater shortening velocity and a greater energetic cost of tension maintenance. The possibility arises that such a relation might exist also in different contractile states of an individual muscle. Suggestion has

1 Mean active tension (\bar{P}), O consumption (J_o), lactate production (J_{LA}) and calculated ATP turnover (J_{ATP}) = $6.42 \times \bar{P} + 1.25 \times J_{LA}$ (cf Peterson & Paul 1974) at long (L) and short (S) muscle length
 = normal tri-buffered medium K-tris = H-high (100 mM) hypertonic solution. Ca^{2+} -concentrations as indicated.
 portal vein was exposed to in sequence. a-tris (2.5 mM Ca^{2+}), w-tris (0 mM Ca^{2+}) and either K-tris or hypertonic solution. a-tris data collected from both groups, but not including such data from muscles used with NaCl-hypertonic solution
 *** = significant ($p < 0.05$, 0.01, 0.001 resp.) difference between results at long and short muscle length as obtained by Student's *t*-test for paired data

medium	[Ca^{2+}] (mM)	Muscle length	\bar{P} (mN/mm ²)	J_o (μ mol/min g)	J_{LA} (μ mol/min g)	J_{ATP} (μ mol/min g)	
norm	2.5	L	2.5 ± 0.4	0.57 ± 0.05	0.22 ± 0.05	3.9 ± 0.3	12
		S	0	0.41 ± 0.03 **	0.33 ± 0.04	2.9 ± 0.2	
	0	L	0	0.39 ± 0.02	0.13 ± 0.02	2.6 ± 0.2	12
		S	0	0.38 ± 0.03	0.14 ± 0.03	2.6 ± 0.2	
m	0.5	L	6.3 ± 1.1	0.48 ± 0.06	0.29 ± 0.08	3.4 ± 0.4	11
		S	0	0.43 ± 0.05	0.15 ± 0.03	2.9 ± 0.3	
300 mOsm sucrose	2.5	L	6.2 ± 0.3	0.46 ± 0.03	0.69 ± 0.08	3.8 ± 0.3	6
		S	0	0.39 ± 0.03	0.61 ± 0.06	3.2 ± 0.3	
at 300 mOsm NaCl	5	L	7.2 ± 0.8	0.51 ± 0.01	0.65 ± 0.04	4.1 ± 0.1	4
		S	0	0.34 ± 0.01	0.59 ± 0.03	2.9 ± 0.1	

ocular scale. The muscle was then removed, blotted, weighed on a Cahn electrobalance. Metabolic data are expressed relative to the tissue wet weight. Force values at lengths are normalized to cross-sectional area, which is calculated from optical length and wet weight, assuming uniform thickness and density of 1.05 mg/mm³.

RESULTS

Table 1 shows measured mean active tension (\bar{P}), oxygen consumption (J_o), lactate production (J_{LA}) and calculated ATP turnover (J_{ATP}) of Peterson & Paul (1974) in portal veins. The muscles at two different lengths, were either kept in normal solution with or without Ca^{2+} or contracted by K or hypertonicity. In normal solution the greater active tension development at long length (L) measured as mean tension (\bar{P}) during spontaneous activation was accompanied by increased J_o and J_{ATP} . Increases J_{LA} in this study was not different from that at short length (S) in normal solution without Ca^{2+} where the muscle was relaxed, no differences in metabolic fluxes were seen between the two lengths. In H-high solution (0.5 mM Ca^{2+}) where a contracture (HC) develops J_o , J_{LA} and J_{ATP} were greater at the long length, but when total rates are compared as in Table 1 this difference is significant only in J_{ATP} . Hypertonicity contractures (HC) are produced by two different agents, sucrose and NaCl. Tension development in both media were about the same, and similar to that in KC. In

HC, all metabolic fluxes increased with tension, although in the total rates this difference was significant in J_o and J_{ATP} only. J_{LA} values were much increased compared to the rates measured in isotonic solutions. This increase was similar for both agents used in producing hypertonicity.

A better resolution of the tension-dependent rates of metabolism was desired than that apparent from the total rates in Table 1. The J_o , J_{LA} and J_{ATP} measured in Ca^{2+} free solution were subtracted from those in the activated muscle at corresponding length. The resulting quantities called here ΔJ_o , ΔJ_{LA} and ΔJ_{ATP} are shown plotted against mean active tension \bar{P} in Fig. 1. HC data for sucrose only are shown since NaCl data were similar. The increase in ΔJ_o from short to long muscle length is significant for all modes of activation. The $\Delta J_o/\bar{P}$ slope is much steeper in normal tri than in HC and KC. At short length ΔJ_o in HC is smaller than in KC and in fact, it is negative. At the long length, ΔJ_o in HC is lower than in KC but the $\Delta J_o/\bar{P}$ slope is about the same. The much greater ΔJ_{LA} in HC compensates for the difference in ΔJ_o so that ΔJ_{ATP} values, both at long and short muscle length, are about the same in HC and KC. It is notable that the tension dependence of ΔJ_{LA} for all modes of activation is weak, since there were no significant differences in ΔJ_{LA} between the muscle lengths.

The increased J_{LA} in the hypertonic medium is a prominent finding, and it was considered interest-

been made of such an effect in the portal vein when phasic and "tonic" activation is compared (Hellstrand 1977, 1979). It would thus be of interest to know whether the low shortening speed in hypertonic medium has a counterpart in a low rate of energy turnover.

In the present investigation we have measured oxygen consumption and lactate production of the rat portal vein during exposure to hyperosmotic solution (about twice normal osmolality) under simultaneous recording of isometric tension. A comparison was made of the energetic aspects of the contractures developed in this medium and in K⁺ high isotonic medium. In addition a comparison with the energetics of spontaneous phasic activity in normal medium was obtained. The results indicate that whereas the lactate production is greatly enhanced in the hyperosmotic solution, oxygen consumption is slightly reduced, so that the overall ATP-production seems to be about the same as in the K⁺-contracture at normal tonicity. It was not possible to show any difference in the cost of contraction between the contractures in hypertonicity and isotonicity.

METHODS

Portal veins from male Sprague-Dawley rats (200–400 g) were dissected and mounted in a glass chamber equipped with an O₂ electrode and a stirrer as described by Hellstrand (1977). In the present series of experiments the perspex plug sealing the chamber was replaced by one made of glass of similar shape. Furthermore the muscle was now fixed to the tension transducer via a fine glass capillary instead of a silk thread. No difference in performance of the apparatus was noted as a result of these modifications of the technique. The chamber could be perfused by solution or sealed for measurement of O₂ consumption. In the present experiments the perfusion rate was about 0.3 ml/min. Since the chamber volume was 1.3 ml this means that at constant rate of lactate production steady state lactate concentration was achieved in about 15 min (see Hellstrand 1977). Samples of the perfusate were collected and assayed for lactate by the fluorimetric method of Lowry & Passonneau (1972) using an Aminco-Bowman spectrophotofluorometer. This method is more sensitive than the previously used spectrophotometric method and allowed a higher flow rate to be used.

The solutions used were as follows: (1) normal tris solution of composition in mM: NaCl 120, KCl 6.0, MgCl₂ 1.0, CaCl₂ 0.5, glucose 11.5, Na₂Ca-versenate 0.026 and tris-(hydroxymethyl)aminomethane (Trizma Base, Sigma Chemical Co.) 23, titrated with HCl to a pH of 7.4 at 37°C. (2) K⁺-high tris of composition as normal tris except that 100 mM of NaCl had been replaced by KCl and that the concentration of CaCl₂ was 0.5 mM. (3) nominal-

ly Ca²⁺-free tris obtained by omitting CaCl₂ in the normal tris. (4) hyperosmotic solutions obtained by sucrose, mannitol or NaCl to the normal or Ca²⁺-free tris. Osmolality was routinely checked. Advanced Instruments Inc. freezing point oven (31-LAS) and was about 790 mosm/kg in the case 390 mosm/kg in the hypertonic media. All solutions bubbled with air prior to entry into the chamber. The temperature of the system was kept at 37°C. To bacterial contamination penicillin G (100 mg/l) and tomycin (300 mg/l) were added to the solution. Na-salts. After the muscle had been mounted in a chamber at a preload of about 5 mN it was left to equilibrate in normal tris under perfusion for 60 min.

A conditioning 30 min exposure to the hyperosmotic medium with 2.5 mM Ca²⁺ was made resulting in slowly developing contracture (HC). Thereafter the protocol was as follows: (1) normal tris for 45 min. (2) nominally Ca²⁺-free tris for 30 min. (3) hyperosmotic medium (2.5 mM Ca²⁺) for 30 min. This protocol was followed in two different lengths of the muscle: one longer at a tension of about 5 mN (optimal for tension development) and one shorter where the muscle developed no tension. In half of the experiments the muscle was kept at longer length first and then shortened, whereas in the other half the sequence was reversed. To ensure an exchange of solution, flow was kept higher (0.8 ml/min) for the initial 5 min in each solution. The measurements of lactate production (J_{LA}) and O₂ consumption (J_{O_2}) were made during the final 15 min in each solution. During this period the perfusate was first collected for 5 min for lactate assay. Then the flow was stopped during the final 10 min the chamber was kept closed for measurement (see Hellstrand 1977). Isometric tension was measured during this period as reported. Blood samples taken from the inflow solution at times corresponding to the lactate samples. The samples and blanks were frozen until analysis. In experiments involving K⁺-contracture (KC) the above protocol was followed, except that high tris replaced the hyperosmotic solution. A section of the protocol where the period in normal tris (above) was omitted was followed for a series of experiments where HC was induced by NaCl instead of by sucrose.

The influences of Ca²⁺ ions and glucose were evaluated in experiments where HC was induced by the addition of NaCl. In these experiments the muscles were kept at optimal length throughout.

In a separate study the variations of J_{O_2} and J_{LA} during 30 min periods of KC or HC was followed. A series of experiments contractures (KC or HC) were made at two different muscle lengths with preceding development of J_{O_2} in Ca²⁺-free solution. The chamber was closed and isometric tension and J_{O_2} were recorded. Records were also stored on magnetic tape (Racal Data Store 4 Instrumentation recorder) for later computer analysis (Alpha-LSI minicomputer). Data from the first 15 min in each contracture (including solution exchange) were discarded and the rest of the period was divided into 5 min intervals of 5 min each. Mean tension and J_{O_2} and J_{LA} interval were calculated by the computer.

At the end of each experiment the muscle was returned to optimal length which was measured by microscope

1. Mean active tension (\bar{P}) O₂ consumption (\dot{V}) lactate production (J_{LA}) and calculated ATP turnover (J_{ATP}) = $6.42 \times \dot{V}_{O_2} + 1.25 \times J_{LA}$ (cf Peterson & Paul 1974) at long (L) and short (S) muscle length
 = normal tris-buffered medium, K⁺-tris = E⁻-high (100 mM) isotonic solution. Ca²⁺-concentrations as indicated
 portal vein was exposed so, in sequence, n-tris (2.5 mM Ca²⁺), n-tris (0 mM Ca²⁺) and either K⁺-tris or hyper-
 tonic solution. n-tris data collected from both groups, but not including such data from muscles used with NaCl-
 hypertonic solution. ** = significant ($p < 0.05$, 0.01, 0.001 resp.) difference between results at long and short
 muscle length as obtained by Student's *t*-test for paired data

ion	[Ca ²⁺] (mM)	Muscle length	\bar{P} (mN/mm ²)	\dot{V}_{O_2} (μ mol/min g)	J_{LA} (μ mol/min g)	J_{ATP} (μ mol/min g)	
K ⁺	2.5	L	2.5 \pm 0.4	0.57 \pm 0.03	0.22 \pm 0.05	3.9 \pm 0.3	12
		S	0	0.41 \pm 0.03 **	0.33 \pm 0.04	2.9 \pm 0.2	
	0	L	0	0.39 \pm 0.02	0.13 \pm 0.02	2.6 \pm 0.2	12
		S	0	0.38 \pm 0.03	0.14 \pm 0.03	2.6 \pm 0.2	
n	0.5	L	6.3 \pm 1.1	0.45 \pm 0.06	0.29 \pm 0.08	3.4 \pm 0.4	6
		S	0	0.43 \pm 0.05	0.15 \pm 0.03	2.9 \pm 0.3	
	300 mOsm sucrose	L	6.1 \pm 0.3	0.46 \pm 0.03	0.69 \pm 0.08	3.8 \pm 0.3	6
		S	0	0.39 \pm 0.03	0.61 \pm 0.06	3.2 \pm 0.3	
Cl ⁻	2.5	L	7.2 \pm 0.6	0.51 \pm 0.01	0.65 \pm 0.04	4.1 \pm 0.1	4
		S	0	0.34 \pm 0.01 **	0.59 \pm 0.02	2.9 \pm 0.1	

sectional area. The muscle was then removed, blotted
 and weighed on a Cahn electrobalance. Metabolic data are
 referred relative to the tissue wet weight. Force values at
 lengths are normalized to cross sectional area. Each
 value calculated from optimal length and wet weight, assum-
 ing uniform thickness and density of 1.05 mg/mm³.

RESULTS

Table 1 shows measured mean active tension (\bar{P}),
 oxygen consumption (\dot{V}), lactate production (J_{LA})
 and calculated ATP turnover (J_{ATP}) of Peterson &
 Paul (1974) in portal veins. The muscles, at two
 different lengths were either kept in normal sol-
 ution, with or without Ca²⁺ or contracted by K⁺ or
 hypertonicity. In normal solution the greater ac-
 tive tension development at long length (L) meas-
 ured as mean tension (\bar{P}) during spontaneous activa-
 tion was accompanied by increased \dot{V}_{O_2} and J_{ATP} ,
 whereas J_{LA} in this study was not different from that
 at short length (S). In normal solution without Ca²⁺
 where the muscle was relaxed, no differences in
 metabolic fluxes were seen between the two
 lengths. In E⁻-high solution (0.5 mM Ca²⁺) where a
 contracture (KC) develops, \dot{V}_{O_2} , J_{LA} and J_{ATP} were
 greater at the long length, but when total rates are
 compared, as in Table 1, this difference is signif-
 icant only in J_{ATP} . Hypertonicity contractures
 (HC) were produced by two different agents: suc-
 rose and NaCl. Tension development in both media
 were about the same and similar to that in KC. In

HC all metabolic fluxes increased with tension,
 although in the total rates this difference was sig-
 nificant in \dot{V}_{O_2} and J_{ATP} only. J_{LA} values were much
 increased compared to the rates measured in
 isotonic solutions. This increase was similar for
 both agents used in producing hypertonicity.

A better resolution of the tension-dependent rates
 of metabolism was desired than that apparent from
 the total rates in Table 1. The \dot{V}_{O_2} , J_{LA} and J_{ATP}
 measured in Ca²⁺-free solution were subtracted
 from those in the activated muscle at corresponding
 length. The resulting quantities, called here $\Delta\dot{V}_{O_2}$,
 ΔJ_{LA} and ΔJ_{ATP} are shown plotted against mean
 active tension \bar{P} in Fig. 1. HC data for sucrose only
 are shown, since NaCl data were similar. The in-
 crease in $\Delta\dot{V}_{O_2}$ from short to long muscle length is
 significant for all modes of activation. The $\Delta J_{ATP}/\bar{P}$
 slope is much steeper in normal tris than in HC and
 KC. At short length ΔJ_{ATP} in HC is smaller than in
 KC and in fact it is negative. At the long length,
 $\Delta\dot{V}_{O_2}$ in HC is lower than in KC but the $\Delta\dot{V}_{O_2}/\bar{P}$
 slope is about the same. The much greater ΔJ_{LA} in
 HC compensates for the difference in $\Delta\dot{V}_{O_2}$ so that
 ΔJ_{ATP} values both at long and short muscle length,
 are about the same in HC and KC. It is notable that
 the tension dependence of ΔJ_{LA} for all modes of
 activation is weak since there were no significant
 differences in ΔJ_{LA} between the muscle lengths.

The increased \dot{V} in the hypertonic medium is
 prominent finding, and it was considered of interest

Table 2. Isometric tension and metabolic fluxes in *L. osmotus* and *L. hyperboreus* during contraction in glucose

Significance levels as in Table 1. In each group, 2 muscles were exposed first to glucose-free and then to glucose-containing media, whereas the 2 other muscles were treated in the reverse order.

Solution	[Ca ²⁺] (mM)	[glucose] (mM)	P (mN/mm ²)	J (μmol/min/g)	J _{rel} (μmol/min/g)	n
n-tris	0	11.5	0	0.27±0.02	0.11±0.03	4
	0	0	0	0.30±0.04	0.02±0.02	4
n-tris+300 mM mannitol	2.5	11.5	6.5±1.0	0.32±0.02	0.49±0.05	4
	2.5	0	8.3±1.5	0.27±0.01	0.02±0.03	4

ing to see what effect omission of glucose in the medium would have in this situation. In Table 2 are summarized 4 experiments where HC is compared to the relaxed (Ca²⁺-free) state in the presence and absence of glucose. To avoid possible glucose contamination HC was produced by mannitol rather than by sucrose. Tension development in HC was not dependent on presence of glucose. J_{rel} was essentially zero in glucose-free solution both in the relaxed and contracted muscle. A slight, non-significant increase in J in glucose-free solution was seen in both mechanical states.

The effects of nominally Ca²⁺-free solution on tension and metabolic fluxes in HC are shown in Table 3. Tension development in HC was not dependent on external Ca²⁺ as already observed (Andersson et al. 1974). In HC J and J_{rel} were unchanged whether Ca²⁺ was present or not.

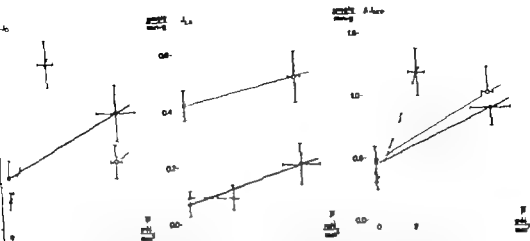
The results presented so far were all obtained from measurements made late during long-standing contractures (30 min). It was considered possible that the metabolic flux rates might be different earlier in the contractures. With the technique used the time resolution of the J_{rel} measurements was not adequate to bear on this question. J_{rel} however can be followed with accuracy during the course of the contraction (see Methods). Fig. 2 shows data on the temporal variations in mean tension (P) and J

during 30 min contractures, divided into five 6 s intervals. Time zero is taken to be about 5 min after the initiation of contraction by solution change. Experiments were done on muscles in HC and in HC-sponges. The left panel shows force at long and short muscle lengths (open and closed circles). Circles indicate HC and squares HC. The difference in length was the same in both kinds of fish (short=40% of long appendage) but whereas there was no tension development at short length in fish some tension was seen in HC. HC volume increase during the exposure period whereas J_{rel} declines from an initial high tension. The J_{rel} values are shown in the middle panel. In HC at long length J_{rel} declines with time whereas at short length it is constant. In HC however J declines with time both at long and short length despite the increase in tension. The tension dependence of J_{rel} is evaluated by computing the quotient (J_{rel} - J_{rel}) / (P_{rel} - P_{rel}) where the superscript L and S indicate muscle length. This is shown in the right panel. It is seen that the quotient remains at the same level throughout the contraction in HC, whereas in HC it declines with time. At the end of contraction quotient is seen to be smaller in HC than in HC. Contrast to the data shown in Fig. 1. We have no explanation for this variability, but it is notable that in this series the developed tension (left panel)

Table 3. Isometric tension and metabolic fluxes in *L. osmotus* with and without added Ca²⁺ during long-standing contraction and in *L. hyperboreus* in the presence of Ca²⁺ during contraction

Significance levels as in Table 1. Similar protocol as in Table 2.

Solution	[Ca ²⁺] (mM)	[glucose] (mM)	P (mN/mm ²)	J (μmol/min/g)	J _{rel} (μmol/min/g)	n
n-tris		11.5	0	0.4 ± 0.02	0.11 ± 0.02	4
n-tris+300 mM mannitol		11	8.3 ± 1.1	0.43 ± 0.02	0.77 ± 0.04	4
			8.7 ± 1.3	0.44 ± 0.04	0.72 ± 0.04	4



1 Rates of O_2 consumption (ΔJ_{O_2}), lactate production (ΔJ_{LA}) and calculated ATP production (ΔJ_{ATP}) above in Ca^{2+} -free solution vs. mean active tension (P). Triangles, broken line: normal tris solution (2.5 mM Ca^{2+}); squares, dotted line: low Ca^{2+} solution (0.5 mM Ca^{2+}); circles, solid line: hypertonic solution (sucrose 2.5 mM Ca^{2+}). Symbols: short length, open symbols: long length. Data shown as mean values ± 5 E.

smaller in KC. The results of these expts thus state that in HC the tension-independent J lines with time, whereas in KC it is constant. On the other hand, in KC there is a decrease with time in the tension-dependent J_{LA} .

DISCUSSION

A starting point for this investigation was the assumption that the greatly reduced shortening of the portal vein in hypertonic solution (Jansson et al. 1974; Hellstrand & Arner 1980) should have its counterpart in a reduced metabolic rate of tension development. We chose to compare hypertonicity contracture (HC) with K-contractures (KC) of similar tension, and with the normal spontaneous activity of the portal vein, quantitated as integrated (mean) tension. Values of J_{O_2} and J in spontaneous activity and J_{LA} in K-contractures of the portal vein were reported by Hellstrand (1977). In the present work a sensitive lactate assay was employed which allowed for higher flow rates through the chamber, thus a better time resolution. This enabled us to assess J_{LA} in sustained contractures as well as in steady state spontaneous activity. Generally J_{LA} values found here were lower than those reported earlier and in spontaneous activity no tension dependence of J_{LA} was found (Fig. 1 middle panel) in contrast to the earlier study.

Both in KC and HC greater J_O was found when isometric tension was greater (Fig. 1 left panel). This tension dependence of J_O was similar in HC and KC and much smaller than in spontaneous activity. J_{LA} was at least twice as great in HC as in KC (Table 1). When the total ATP-turnover was calculated according to standard stoichiometry (cf Peterson & Paul 1974) similar values were found in KC and HC, both in absolute amounts and in relation to developed tension (Table 1 and Fig. 1 right panel).

Increased lactate production in hypertonic medium has already been observed in skeletal muscle (Clausen 1968). Possible causes of this effect could be stimulation of glycolysis as a result of increased glucose uptake, accelerated glycogenolysis (Clausen 1968), inhibition of Krebs cycle or respiratory uncoupling. Hypertonicity might be able to stimulate some mechanism preferentially utilizing glycolytically supplied energy. A linkage between ionic pump activity and glycolysis has been suggested (Muller 1964; Pease, Bauer & Paul 1979).

For the interpretation of these results it should be kept in mind that most of our metabolic measurements were performed late in long-standing contractures, whereas in the parallel mechanical expts (Hellstrand & Arner 1980) the K-contractures for technical reasons were kept at only a few min duration. It is possible that force-velocity measurements performed later during a K-contracture would

Table 2 Isometric tension and metabolic fluxes in is osmotic and hyperosmotic solutions with and without glucose

Significance levels as in Table 1. In each group 2 muscles were exposed first to glucose free and then to glucose containing media whereas the other muscles were treated in the reverse order.

Solution	[Ca ²⁺] (mM)	[glucose] (mM)	P (mN/mm ²)	J (μ mol/min x g)	J (μ mol/min x g)	
n-tris	0	11.5	0	0.77 \pm 0.0	0.17 \pm 0.03	4
	0	0	0	0.30 \pm 0.04	0.02 \pm 0.02*	4
n-tris + 300 mOsm	5	11.5	6.8 \pm 1.0	0.3 \pm 0.07	0.49 \pm 0.05	4
mannitol	7.5	0	8.3 \pm 1.5	0.17 \pm 0.01	0.0 \pm 0.01	4

ing to see what effects omission of glucose in the medium would have in this situation. In Table 2 are summarized 4 expts where HC is compared to the relaxed (Ca²⁺ free) state in the presence and absence of glucose. To avoid possible glucose contamination HC was produced by mannitol rather than by sucrose. Tension development in HC was not dependent on presence of glucose. J_{L_1} was essentially zero in glucose-free solution both in the relaxed and contracted muscle. A slight non significant increase in J_{L_1} in glucose-free solution was seen in both mechanical states.

The effects of nominally Ca²⁺ free solution on tension and metabolic fluxes in HC are shown in Table 3. Tension development in HC was not dependent on external Ca²⁺ as already observed (Andersson et al 1974). In HC J_{L_1} and L_1 were unchanged whether Ca²⁺ was present or not.

The results presented so far were all obtained from measurements made late during long-standing contractures (30 min). It was considered possible that the metabolic flux rates might be different earlier in the contractures. With the technique used the time resolution of the J_{L_1} measurements was not adequate to bear on this question. J_{L_1} however can be followed with accuracy during the course of the contraction (see Methods). Fig. 2 shows data on the temporal variations in mean tension (\bar{P}) and J_{L_1}

during 30 min contractures divided into five 6 s intervals. Time zero is taken to be about 5 min after the initiation of contraction by solution change. expts were done in 3 involving KC and 6 HC responses. The left panel shows force at long and short muscle lengths (open and closed symbols). Circles indicate KC and squares HC. The difference in length was the same in both kinds of expts (short = 40% of long approx.) but whereas there was no tension development at short length in KC some tension was seen in HC. HC contractures increase during the exposure period whereas KC declines from an initial high tension. The J_{L_1} are shown in the middle panel. In KC at long length J_{L_1} declines with time whereas at short length it is constant. In HC however J_{L_1} declines with time both at long and short length despite the increase in tension. The tension dependence of J_{L_1} is evaluated by computing the quotient ($J_{L_1}^L / J_{L_1}^S$) ($P^L - P^S$) where the superscripts L and S indicate muscle length. This is shown in the right panel. It is seen that the quotient remains at the same level throughout the contraction in HC whereas in KC it declines with time. At the end of contraction the quotient is seen to be smaller in KC than in HC. contrast to the data shown in Fig. 1. We have a explanation for this variability but it is notable that in this series the developed tension (left panel)

Table 3 Isometric tension and metabolic fluxes in is osmotic Ca²⁺ free solution and in hyperosmotic solution with and without added Ca

Significance levels as in Table 1. Similar protocols as in Table 2.

Solution	[Ca ²⁺] (mM)	[glucose] (mM)	P (mN/mm ²)	J (μ mol/min x g)	J (μ mol/min x g)
n-tris	0	11.5	0	0.42 \pm 0.02	0.11 \pm 0.02
n-tris + 300 mOsm	5	11.5	8.3 \pm 1.1	0.43 \pm 0.02	0.77 \pm 0.04
mannitol	0	11.5	8.7 \pm 1.3	0.44 \pm 0.04	0.77 \pm 0.04

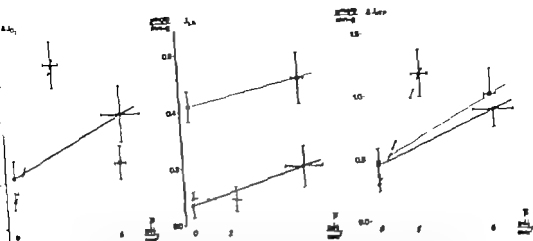


Fig. 1. Rates of O_2 consumption (ΔJ_{O_2}), lactate production (ΔJ_{LA}) and calculated ATP production (ΔJ_{ATP}) above a Ca^{2+} -free solution vs. isometric tension (P). Triangles, broken line: normal Ca^{2+} solution ($1.5 \text{ mM } Ca^{2+}$). Squares, dotted line: hypertonic solution (sucrose $2.5 \text{ mM } Ca^{2+}$). Filled symbol: short length, open symbol: long length. Data shown as mean values \pm S.E.

smaller in KC. The results of these expts thus state that in HC the tension-independent J_0 increases with time, whereas in KC it is constant. On the other hand, in KC there is a decrease with time of the tension-dependent J .

DISCUSSION

Starting point for this investigation was the assumption that the greatly reduced shortening of the portal vein in hypertonic solution (Hellsing et al. 1974; Hellsing & Arner 1980) could have its counterpart in a reduced metabolic of tension development. We chose to compare hypertonicity contracture (HC) with K⁺-induced contractures (KC) of similar tension, and with the normal spontaneous activity of the whole muscle, quantitated as integrated (mean) values of J_0 and J in spontaneous activity and J_0 in K⁺-contractures of the portal vein were reported by Hellsing (1977). In the present work a more sensitive lactate assay was employed, which allowed for higher flow rates through the chamber than a better time resolution. This enabled us to study J_{LA} in so termed contractures as well as in steady state spontaneous activity. Generally J_{LA} values found here were lower than those reported here and in spontaneous activity no tension dependence of J_{LA} as found (Fig. 1 middle panel) in contrast to the earlier study.

Both in KC and HC greater J was found when isometric tension was greater (Fig. 1 left panel). This tension dependence of J_0 was similar in HC and KC and much smaller than in spontaneous activity. J_{LA} was at least twice as great in HC as in KC (Table 1). When the total ATP turnover was calculated according to standard stoichiometry (cf Peterson & Paul 1974) similar values were found in KC and HC both in absolute amounts and in relation to developed tension (Table 1 and Fig. 1 right panel).

Increased lactate production in hypertonic medium has already been observed in skeletal muscle (Chausen 1968). Possible causes of this effect could be stimulation of glycolysis as a result of increased glucose uptake, accelerated glycolysis (Chausen 1968), inhibition of Krebs cycle or respiratory uncoupling. Hypertonicity might be able to stimulate some mechanism preferentially utilizing glycolytically supplied energy. A linkage between ionic pump activity and glycolysis has been suggested (Müller 1962; Pease, Bauer & Paul 1979).

For the interpretation of these results it should be kept in mind that most of our metabolic measurements were performed late in long-standing contractures, whereas in the parallel mechanical expts (Hellsing & Arner 1980) the K⁺-contractures for technical reasons were kept at only a few min duration. It is possible that force velocity measurement performed later during K⁺-contracture would

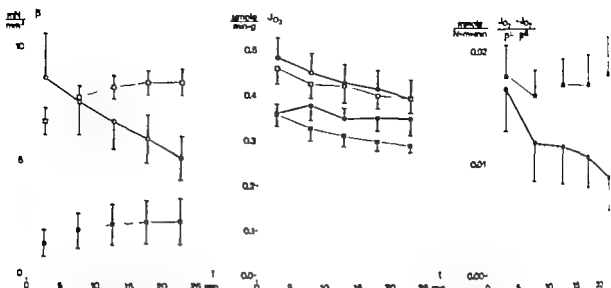


Fig. 2. Variation in developed tension (\bar{P}), O_2 consumption (J_{O_2}) and tension-related O_2 consumption [$J_{\text{O}_2}/(\bar{P}_i - \bar{P}_a)$] during the course of 30 min contractions at short (S) and long (L) muscle lengths. Circles, full Ca^{2+} high solution (0.5 mM Ca^{2+}); squares, dotted line, hyperosmotic solution (sucrose 3.5 mM Ca^{2+}). Full symbols, short length; open symbols, long length. Data shown as mean values \pm S.E., 6 experiments in each group.

have given a lower maximal shortening velocity. Results indicating such an effect in amphibian slow twitch fibres have been reported by Lännergren (1978). However, the difference in shortening velocity between hC and HC of the portal vein is so great (about 5-fold) that it appears unlikely to be entirely abolished even when measurements are made late in contractions of both kinds. It is to be noted that the tension-related J_{O_2} seems to diminish with time in hC in contrast to HC (Fig. 2, right panel).

The tension development in HC occurs independent of membrane potential and at levels of extracellular $[\text{Ca}^{2+}]$ unable to sustain a hC (Andersson et al. 1974; McGrath & Shepherd 1976; Hellstrand & Arner 1980). Work on skeletal muscle suggests that hypertonic solutions may mobilize Ca^{2+} ions from intracellular stores and also increase membrane permeability for Ca^{2+} (Yamada 1970; Lännergren & Noth 1973; Homsher, Briggs & Wise 1974; Clausen, Dahl, Hansen & Elbrink 1979). It is also notable that Ca^{2+} accumulation by isolated smooth muscle mitochondria is inhibited in hyperosmotic solution (S. Batra, personal communication). Thus, it is possible that at least part of the tension increase in the portal vein in hyperosmotic solution is due to activation of the contractile proteins by elevated $[\text{Ca}^{2+}]$. At lower degrees of hyperosmolality (below 100 mosm/l) an increase is seen in peak force of portal vein spontaneous con-

tractions (McKinley, McKenzie & Blair-West 1974) and hC-contractions (S. Sigurdsson, personal communication).

The maximal tension development of a hC in the portal vein is less than that of a hC in isotonic solution at optimal Ca^{2+} -concentration. Experiments on isolated skeletal muscle reveal decreased tension development at increased ionic strength (Gordon 1973; Homsher et al. 1974). At high ionic strength, actomyosin ATPase of vascular smooth muscle is decreased (Maxwell, Bohr & Murphy 1971). Depression of the maximal contractile activation, the changes in stiffness and shortening velocity caused by hypertonic solutions seem to be separable from the contractile activation and the apparent increase of cytoplasmic Ca^{2+} . It is not clear whether HC is entirely caused by Ca^{2+} -dependent mechanisms (increased Ca^{2+} level and/or more Ca^{2+} sensitivity of the contractile system) or if, after prolonged depletion of Ca^{2+} , about 40% of control HC response can be elicited (Andersson et al. 1974). The possibility of activation independent of Ca^{2+} is considered in the concomitant paper (Hellstrand & Arner 1980).

The results of this study do thus not support the hypothesis that the low maximal shortening velocity of the contracture in the hypertonic medium has a counterpart in a lower metabolic cost of tension. Rather, the results seem to be comparable with a situation in the hypertonic medium where

ed number of cross-bridges operate in an usually normal way. The possibilities of rigor linkages and/or ultrastructural alterations in the active apparatus in hypertonicity have been used (Hellstrand & Arner 1980). Such factors reduce the number of active cross-bridges and use an internal resistance in shortening.

would like to thank Monica Lendahl, Monica Eriksson and Lea Nordström for technical assistance. Investigation was supported by grants from the Swedish Medical Research Council (0-01-0028), the Royal Faculty University of Lund and AB Hjälsjö, Borås.

REFERENCES

- ERIKSSON C, HELLSTRAND P, JOHANSSON A & RINGBERG A. 1974 Contraction in venous smooth muscle induced by hypertonicity. Calcium dependence and mechanical characteristics. *Acta Physiol Scand* 90 451-461.
- LYSEN T. 1968 The relationship between the transport of glucose and cations across cell membranes in isolated tissues. III. Effect of Na⁺ and hyperosmolarity on glucose metabolism and insulin responsiveness in isolated rat hepatocytes. *Biochim Biophys Acta* 150 56-65.
- LYSEN T, DAHL-HANSEN A B & ELBRINK, 1979 The effect of hyperosmolarity and insulin on resting tension and calcium fluxes in rat soleus muscle. *J Physiol (Lond)* 292 505-526.
- LYSEN T, A. M., GODT R E., DONALDSON S K. & HARRIS C E. 1973 Tension in skinned frog muscle fibers in solutions of varying ionic strength and several salt compositions. *J Gen Physiol* 61 553-574.
- HELLSTRAND P. 1977 Oxygen consumption and lactate production of the rat portal vein in relation to its contractile activity. *Acta Physiol Scand* 100 91-106.
- HELLSTRAND, P. 1979 Mechanical and metabolic properties related to contraction in smooth muscle. *Acta Physiol Scand*, Suppl. 464 1-34.
- HELLSTRAND P & ARNER, A. 1980 Contraction of the rat portal vein in hypertonic and isotonic medium: mechanical properties and effects of Mg²⁺. *Acta Physiol Scand* 110 59-67.
- HOMSHER, E., BRIGGS, F N & WISE, R. M. 1974 Effects of hypertonicity on resting and contracting frog skeletal muscles. *Amer J Physiol* 226 835-843.
- HOWARTH, J V. 1958 The behaviour of frog muscle in hypertonic solutions. *J Physiol (Lond)* 144 167-175.
- JEWELL, B. R. & WILKIE, H R. 1958 An analysis of the mechanical components in frog striated muscle. *J Physiol (Lond)* 143 515-540.
- LÄNNERGREN J. 1978 The force-velocity relation of isolated (twitch and slow muscle fibres of xenopus larvae. *J Physiol (Lond)* 283 501-521.
- LÄNNERGREN J & NOTH, J. 1973 Tension in isolated frog muscle fibers induced by hypertonic solutions. *J Gen Physiol* 61 158-175.
- LOWRY O H & PASSONNEAU J V. 1972 A flexible system of enzymatic analysis. Academic Press, New York & London.
- MORATH M. A. & SHEPHERD J T. 1976 Hyperosmolarity effects on nerves and smooth muscle of cutaneous veins. *Amer J Physiol* 231 141-147.
- McKINLEY M J, McKENZIE, J S. & BLAIR WEST J R. 1974 Effects of maintained osmolarity changes on rat portal vein spontaneous contractions. *Amer J Physiol* 226 718-723.
- MAXWELL, L C, BOHR, D F & MURPHY R. A. 1971 Arterial actomyosin: effects of ionic strength on ATPase activity and solubility. *Amer J Physiol* 220 1871-1874.
- MULLER, M. H. 1962 Metabolic aspects of ionic shifts in toad muscle. *Biochim Biophys Acta* 57 475-494.
- PEASE, W, BAUER, M. & PAUL, R. J. 1979 Anaerobic aerobic glycolysis in vascular smooth muscle (VSM) is coupled to Na-K transport processes. *Biophys J* 25 151a.
- PETERSON J W & PAUL, R. J. 1974 Aerobic glycolysis in vascular smooth muscle: relation to isometric tension. *Biochim Biophys Acta* 357 167-176.
- RÜEGG J C. 1971 Smooth muscle tone. *Physiol Rev* 51 201-48.
- YAMADA K. 1970 The increase in the rate of heat production of frog skeletal muscle caused by hyperosmotic solutions. *J Physiol (Lond)* 208 49-64.

Reflex inhibition of sympathetic activity during volume load in awake normotensive and spontaneously hypertensive rats

RICKSTEN and P. THOREN

Department of Physiology, University of Göteborg, Sweden

RICKSTEN S.-E. & THOREN P. Reflex inhibition of sympathetic activity during volume load in awake normotensive and spontaneously hypertensive rats. *Acta Physiol Scand* 1980; 110: 77-82. Received 17 Dec 1979. ISSN 0001-6772. Department of Physiology, University of Göteborg, Sweden.

The reflex inhibition of the sympathetic activity in the splanchnic nerves was recorded upon volume expansion with blood in awake spontaneously hypertensive rats (SHR) and in normotensive Wistar-Kyoto rats (WKR) at an age of 16-20 weeks. At 10% blood volume expansion SHR showed significantly greater nerve inhibition (43%) in comparison with WKR (33%). This augmented reflex response was not caused by the arterial baroreceptors, because the sensitivity of the arterial baroreceptor reflex arch, if anything, tended to be lower in SHR and the increase in arterial blood pressure upon volume load was also lower in SHR. It is suggested that the reason for this increased reflex inhibition in SHR is an augmented low pressure receptor response. The mechanism behind this is discussed. The most likely explanation is decreased distensibility of the venous system, the systemic and/or the pulmonary veins.

Key words: Awake rats, sympathetic nerve recording, SHR, WKR, volume load.

Intrathoracic low pressure mechanoreceptors of great importance for the reflexogenic control of the cardiovascular system. There are two types of receptor endings in the cardiopulmonary region: afferent endings in the vagal nerves. One type of receptor-endings is mainly located at the junction of the great veins with the right and left atria. These atrial receptors have rapidly conducting myelinated afferent. Upon activation these receptors evoke reflex tachycardia and water diuresis (Andersson 1976, Thoren 1979). The large population of receptor-endings with nonmyelinated afferent are located both in the atria, pulmonary veins and in the left ventricle (Thoren 1979). Activation of these receptors induces reflex tachycardia and inhibition of sympathetic discharge, especially to the kidneys.

Thoren et al (1979) were the first to study these receptors in genetic hypertension. Using the spontaneously hypertensive rat (SHR) they found that the cardiopulmonary receptors with nonmyelinated afferent are clearly reset. A higher left atrial

pressure (≈ 10 mmHg) was needed to activate the receptors in SHR compared to normotensive control rats (≈ 5 mmHg). Ricksten et al (1979) also studied the reflex inhibition of renal sympathetic nerve activity from the cardiopulmonary receptors in SHR. In anesthetized thoracotomized and baroreceptor denervated rats it was shown that during volume load with plasma given intravenously the nerve activity started to be inhibited at a left atrial pressure around 10 mmHg in SHR in comparison with 5 mmHg in normotensive control rats. Interestingly enough, however, a smaller amount of plasma was needed in SHR to reach the threshold left atrial pressure for reflex inhibition of the nerve activity compared to normotensive control rats. In other words, the low pressure receptors with nonmyelinated afferents (mainly located in the left atrium in the rat, Thoren et al 1979) seem to be activated more for a given increase of the plasma volume in SHR compared to normotensive control rats, but a higher pressure was also needed. As, however, the animal in the mentioned study were

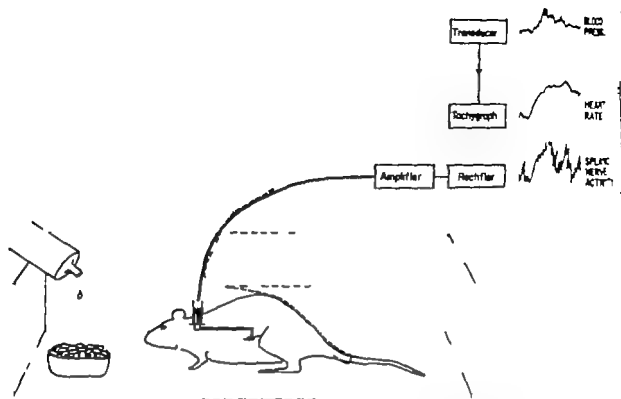


Fig. 1. Schematic drawing of the preparation. The experiments were performed with the animals in a cage to which they had been habituated for 36 h before the experiments, and where they were provided with food and water.

thoracotomized and the surgical trauma was rather extensive, we wanted to examine the reflex nerve inhibition upon volume load in intact, awake, normotensive and hypertensive animals.

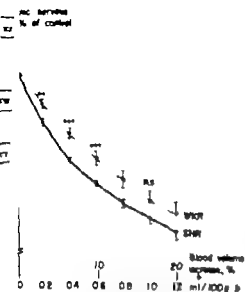
METHODS

Nine spontaneously hypertensive rats (SHR) weighing 335 ± 12 g were used in this study. Eight normotensive rats of the Wistar Kyoto (WKR) strain were used as controls, weighing 342 ± 12 g. Both groups were 4–5 months old. Intraarterial blood pressure was measured in the caudal artery after light ether anesthesia some days before the surgical procedure. Mean arterial pressures in SHR and WKR were 155 ± 4 mmHg and 107 ± 1 mmHg, respectively. During Nembutal® anesthesia the coeliac ganglion and the splanchnic nerves were identified via a left retroperitoneal incision. A thin bipolar silver electrode was placed around a branch of the splanchnic nerve and isolated carefully with silicone rubber (Wacker Sil Gel 604). The animals were then allowed to recover for at least 36 h. Splanchnic nerve activity was recorded via a cable connected to an adaptor on the back of the animal (Fig. 1). The nerve signal was amplified (Grass 511) and rectified and the mean nerve activity was displayed on a Grass Polygraph (model 7). Arterial pressure and heart rate were continuously measured via a catheter placed in the caudal artery (see Fig. 1). The animals were placed in their own cages and received food and water.

In 11 of 17 expts. it was necessary to sedate the animals with diazepam 50 µg i.v. (Valium®) in order to get a proper control level of sympathetic nerve discharge. Though doses of Valium sedated the animals, they could easily be awakened and aroused by e.g. touch stimuli. The administration of Valium did not change the basal level of sympathetic nerve discharge but decreased the endorhelic elicited sympathetic excitations otherwise often seen in these awake animals.

EXPERIMENTAL PROCEDURES

Initially baroreceptor sensitivity was estimated by a i.a. noradrenaline infusion which raised mean arterial pressure around 50 mmHg over ~3 min. The pressure change was plotted against baroreceptor-induced inhibition and baroreceptor sensitivity was defined as percentage nerve inhibition per mmHg of arterial pressure rise. After a control period of about 30 min the animals were then volume loaded i.a. with blood from a rat of the same colony. Splanchnic nerve activity was expressed as per cent of control activity and was then plotted against the degree of blood volume expansion. The volume load was expressed as ml/100 g b.w. and also as per cent blood volume increase. Rippe et al. (1978) have shown that blood volume is about 6 ml/100 g in both SHR and normotensive control rats. Addition of 0.6 ml/100 g of blood therefore corresponds to a blood volume expansion of about 10% in both SHR and WKR. Each experiment was terminated by postmortal nerve activity.



Splenic nerve inhibition during volume load blood in awake SHR and WKR. The change in blood activity is expressed in absolute terms and in percentage ($P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$).

the sympathetic activity which was $0.2-0.9 \mu V$. Group comparison -test was used for the statistical evaluations. value < 0.05 was considered as statistically significant.

RESULTS

Estimated baroreceptor sensitivity was not markedly different between the WKR and the SHR, although it tended to be reduced in SHR, $+0.2 \text{ \%/mmHg}$ compared to 2.0 ± 0.3 in WKR. Fig. 2 shows the mean values of sympathetic inhibition plotted against degree of blood volume expansion. At 10% blood volume load SHR showed a significantly greater nerve inhibition (70%) compared with WKR (75%) while the difference was not significant at more extensive volume expansion. This difference in response between SHR and WKR was not due to an augmented inhibition from the arterial baroreceptors in SHR, because arterial pressure increased indeed less in SHR than in WKR (Fig. 3) upon blood volume load. From the data on baroreceptor sensitivity and changes in arterial pressure the relative contribution of arterial baroreceptors to sympathetic nerve inhibition upon volume load could be estimated (Fig. 4). From the figure it is clear that the relative

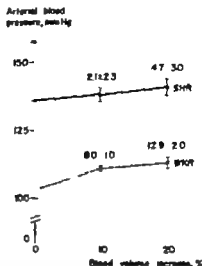


Fig. 3 The change in mean arterial pressure upon volume load in SHR and WKR. The repeated injections of small blood amounts (0.5 ml) were made intraarterially. Note that SHR increased arterial pressure less than WKR for a certain change in blood volume.

contribution of arterial baroreceptors is less in SHR, despite the fact that the degree of sympathetic inhibition is greater in SHR upon 10% volume load. It is likely that cardiopulmonary receptors are responsible for the remaining (non-arterial baroreceptor dependent) inhibition (Ricksten et al 1979; Thoren 1979).

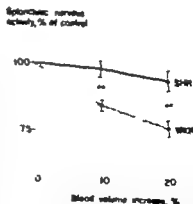


Fig. 4 The estimated contribution of arterial baroreceptors to reflex sympathetic inhibition in response to volume load. The data are obtained from the values of arterial baroreceptor sensitivity (see text) and changes in mean arterial pressure upon volume load (Fig. 3).

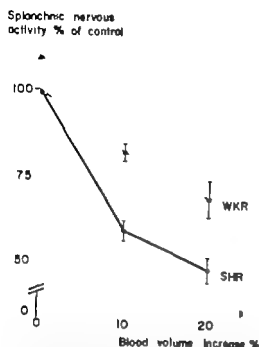


Fig. 5 The estimated contribution of cardiac receptors to reflex sympathetic inhibition in response to volume load. The curve is derived from a subtraction of the values in Fig. 4 from the corresponding values in Fig. 2.

Fig. 5 shows the estimated reflex sympathetic inhibition evoked from cardiopulmonary receptors. This figure is derived from the difference between total sympathetic inhibition (Fig. 2) and that caused by arterial baroreceptors (Fig. 4). Fig. 5 shows that a 10% and 20% volume load activates cardiopulmonary receptors more in SHR leading to an exaggerated reflex inhibition of splanchnic nerve activity in hypertensive animals compared to normotensive ones.

DISCUSSION

The most significant finding in this study is the fact that SHR shows a more pronounced reflex inhibition (Fig. 7) of splanchnic sympathetic activity upon volume load than normotensive controls. Thus, at a 10% increase of blood volume sympathetic discharge decreased significantly more in SHR than in WKR. We here assume that the total blood volume per 100 g b.w. is about the same in SHR and WKR as reported by Rippe et al. (1978). Other investigators report a 12.5% greater blood volume in SHR compared to normotensive Wistar rats (Nikodijevic et al. 1977). If this is true the reflex inhibitory response to a given volume load (ml/100 g) would be even more marked than shown in Fig.

7. In a recent study it is shown that the total volume is 10% greater in WKR compared to S. at an age of 3–4 months (Lundin personal communication). If Fig. 7 is recalculated according to Lundin's data there is still a significantly greater reflex sympathetic inhibition in SHR than at a volume expansion of 10% ($P < 0.01$).

Arterial baroreceptor contribution. What is the reason for this more powerful reflex inhibitory response to volume load in SHR? It is not mediated via arterial baroreceptors because if anything sensitivity of the baroreceptor reflex arc is lower in SHR. Thus, for a given change in pressure SHR tended to inhibit sympathetic activity less than WKR, though this difference was not statistically significant. Such reduced sensitivity of the arterial baroreceptors in SHR have been reported by several previous investigators (de Waele 1976; Brown et al. 1976).

In addition, arterial pressure increased less in SHR upon volume load (Fig. 3). Fig. 4 shows the estimated contribution of arterial baroreceptors to reflex sympathetic inhibition upon volume load in SHR and WKR. These receptors seem to contribute much less in SHR than in WKR because the sensitivity of the baroreceptor reflex arc is decreased in SHR and furthermore the initial arterial pressure was smaller in SHR. We are aware that objections can be raised against such an interpretation as shown in Fig. 4 because the baroreceptor sensitivity is obtained by normoadrenaline infusion and this value for baroreceptor sensitivity is used to estimate the baroreceptor contribution to the reflex sympathetic inhibition upon volume load. In these two situations the pulse pressure is likely to be different also at similar changes in mean arterial pressure. However, the markedly decreased arterial baroreceptor contribution to reflex sympathetic inhibition in response to volume load in SHR is highly likely to be real even when this objection is taken into consideration.

The role of cardiopulmonary receptors. What is the reason for the augmented reflex response to volume load in SHR? A priori the most likely explanation is an augmented low pressure receptor activation. Thus, there exists throughout the cardiopulmonary area a large population of cells with nonmedullated vagal afferents which exert tonic nervous inhibitory influence on outflow from cardiovascular centers (Thoren 1979). The end organs respond to elevations of central blood volume and

to be of great importance for reflex cardiovascular control. These low-pressure volume receptors have been studied recently in normotensive and spontaneously hypertensive rats. In the rat the endings are located mainly in the left atriothorax (Thoren et al 1979a) and they respond markedly to moderate elevation of left atrial pressure, threshold around 5 mmHg in left atrial pressure in normotensive rats. In SHR the receptor threshold is considerably reset, being around 10 mmHg (Thoren et al 1979b).

The present data, showing a hyperreactive volume receptor mechanism in SHR, might seem like a contradiction when related to earlier data on resetting of pulmonary receptors in SHR, as discussed below.

However, the reason for the augmented volume receptor response in SHR is probably a considerable reduction of the capacitance function in the pulmonary system.

Thus Ricksten et al. (1979) plotted the pressure-volume curves against left atrial pressure and found that the pressure-volume load with plasma. Interestingly, the pressure-volume curves in SHR increased more steeply than in normotensive rats. The receptor resetting can thus be offset by the decreased distensibility of the low-pressure capacitance system and changes in both pulmonary and systemic systems may be of importance in this respect. A decreased distensibility of the capacitance system in SHR can either be due to structurally increased stiffness of venous walls, or to increased vasoconstriction due to an overactivity of vasoconstrictor fibres to capacitance vessels, or both.

Simon (1976) showed that the venous pressure-volume curves are shifted to the pressure axis in SHR compared to normotensive control rats indicating decreased venous capacity in SHR. Also Simon and Nilsson (personal communication) noted reduced distensibility of SHR capacitance vessels compared with normotensive control rats at complete relaxation of the vascular bed. In the resting situation SHR shows a considerable elevation of left atrial pressure (Norell et al 1979). A decreased distensibility of the left atrial and of the pulmonary capacitance system are likely to be secondary to the increased pressure in the left atrium and the pulmonary veins. Zanchetti et al. (1978) found that the portal caval vein and the pulmonary artery exhibit marked wall hypertrophy in SHR. Whatever the reason behind the decreased distensibility of systemic and pulmonary veins in SHR, a given volume

load will be more 'centralized' in SHR than in WKR. This will in turn lead to a greater distension of left atrial walls in SHR, to a greater receptor activation and consequently to an accentuated reflex sympathetic inhibition. This, in spite of the assumed decreased distensibility of the left atrial wall in the SHR, which per se should counteract this accentuated reflex response to a volume load. Still, this hypothesis has to be proven.

Physiological significance of augmented volume reflexes in SHR

A surprising finding in the present study was that mean arterial pressure increased less in SHR than in WKR upon 10% blood volume expansion. Thus, the augmented volume receptor reflex response in SHR allows these animals to better cope with increases in blood volume in at least terms of increases in arterial pressure and therefore with less of arterial baroreceptor activation.

Another interesting phenomenon in hypertensive animals and man is the exaggerated natriuretic response to saline load (Wulfsberg et al 1976). It is possible that this is at least partly due to such an accentuated reflex sympathetic inhibition of low-pressure receptors in SHR upon volume loading, because renal sympathetic nerves are known to markedly influence both sodium excretion (DiBona 1977) and renin release (Zanchetti 1976).

REFERENCES

- BROWN A. M., SAUM W. R. & TULEY F. H. 1976. A comparison of aortic baroreceptor discharge in normotensive and spontaneously hypertensive rats. *Circ Res* 39, 688-696.
- DIBONA, G. F. 1977. Neurogenic regulation of renal tubular sodium reabsorption. *Amer J Physiol* 233, F73-F81.
- LINDEN H. J. 1976. Reflex from receptors in the heart. *Cardiology* 61 (Suppl. 1), 7-30.
- NIKOLJEVIC, B., DOLGORA-KORUBIN V. & TADZER, I. S. 1972. Blood volume and spontaneously hypertensive rats. *Acta Med Jugoslav* 26, 251-256.
- NORELL E., RICKSTEN S. E. & THOREN P. 1979. Left atrial pressure in normotensive and spontaneously hypertensive rats. *Acta Physiol Scand* 107, 9-12.
- RICKSTEN S. E., NORELL E. & THOREN P. 1979. Inhibition of renal sympathetic nerve traffic from cardiac receptors in normotensive and spontaneously hypertensive rats. *Acta Physiol Scand* 106, 17-22.
- RIPPE, B., LUNDIN S. & FOLKOW B. 1978. Plasma volume, blood volume and transcapillary escape rate (TER) of albumin in young spontaneously hypertensive rats.

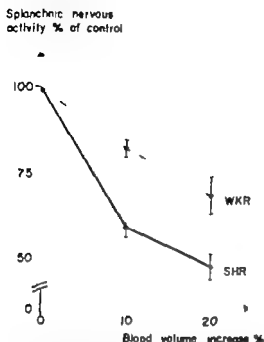


Fig. 5 The estimated contribution of cardiac receptors to reflex sympathetic inhibition in response to volume load. The curve is derived from a subtraction of the values in Fig. 4 from the corresponding values in Fig. 3.

Fig. 5 shows the estimated reflex sympathetic inhibition evoked from cardiopulmonary receptors. This figure is derived from the difference between total sympathetic inhibition (Fig. 3) and that caused by arterial baroreceptors (Fig. 4). Fig. 5 shows that a 10% and 20% volume load activates cardiopulmonary receptors more in SHR leading to an exaggerated reflex inhibition of splanchnic nerve activity in hypertensive animals compared to normotensive ones.

DISCUSSION

The most significant finding in this study is the fact that SHR shows a more pronounced reflex inhibition (Fig. 3) of splanchnic sympathetic activity upon volume load than normotensive controls. Thus at a 10% increase of blood volume sympathetic discharge decreased significantly more in SHR than in WKR. We here assume that the total blood volume per 100 g b.w. is about the same in SHR and WKR as reported by Rippe et al. (1978). Other investigators report a 12.5% greater blood volume in SHR compared to normotensive Wistar rats (Nikodjjevic et al. 1972). If this is true the reflex inhibitory response to a given volume load (ml/100 g) would be even more marked than shown in Fig.

2. In a recent study it is shown that the total blood volume is 10% greater in WKR compared to an age of 3–4 months (Lundin personal communication). If Fig. 2 is recalculated according to Lundin's data there is still a significant reflex sympathetic inhibition in SHR than in WKR at a volume expansion of 10% ($P < 0.01$).

Arterial baroreceptor contribution. What is the reason for this more powerful reflex inhibition response to volume load in SHR? It is not mediated via arterial baroreceptors because of the lower sensitivity of the baroreceptor reflex arc in SHR. Thus for a given change in pressure SHR tended to inhibit sympathetic charge less than WKR though this difference was not statistically significant. Such reduced sensitivity of the arterial baroreceptors in SHR has been reported by several previous investigators (for Wang 1976; Brown et al. 1976).

In addition arterial pressure increases in SHR upon volume load (Fig. 3). Fig. 4 shows the estimated contribution of arterial baroreceptors to reflex sympathetic inhibition upon volume load in SHR and WKR. These receptors seem to contribute much less in SHR than in WKR. Is the sensitivity of the baroreceptor reflex arc decreased in SHR and furthermore the local arterial pressure was smaller in SHR. We are aware that objections can be raised against such a conclusion as shown in Fig. 4 because the baroreceptor sensitivity is obtained by normodistension and this value for baroreceptor sensitivity is used to estimate the baroreceptor contribution to the reflex sympathetic inhibition upon volume load. In these two situations the pulse pressure will be different also at similar changes in arterial pressure. However the markedly decreased arterial baroreceptor contribution to reflex sympathetic inhibition in response to volume load in SHR is highly likely to be real even when this objection into consideration.

The role of cardiopulmonary receptors. What is the reason for the augmented reflex response to volume load in SHR? A priori the most likely explanation is an augmented low pressure receptor activation. Thus there exists throughout the cardiopulmonary area a large population of receptors with nonmedullated vagal afferents which exert tonic nervous inhibitory influence on cardiovascular centers (Thoren 1979). The end organs respond to elevations of central blood volume and

to be of great importance for reflex cardiovascular control. These low-pressure volume receptors have been studied recently in normotensive and spontaneously hypertensive rats. In the rat the endings are located mainly in the left atrium (Thoren et al. 1979a) and they respond markedly to moderate elevation of left atrial pressure, threshold around 5 mmHg in left atrial pressure in normotensive rats. In SHR the receptor load is considerably reset, being around 10 mmHg (Thoren et al. 1979b).

The present data, showing a hyperreactive volume receptor mechanism in SHR, might seem like a contradiction when related to earlier data on resetting of pulmonary receptors in SHR, as discussed above. However, the reason for the augmented volume receptor response in SHR is probably a considerable reduction of the capacitance function in the pulmonary system. Thus, Ricksten et al. (1979) plotted the pressure in plasma volume against left atrial pressure during volume load with plasma. Interestingly enough, left atrial pressure in SHR increased more given volume increase than in normotensive rats. The receptor resetting can thus be offset by a decrease distensibility of the low-pressure capacitance system and changes in both pulmonary and systemic veins may be of importance to this effect. A decreased distensibility of the capacitance system in SHR can either be due to a structurally increased stiffness of venous walls, or to increased vasoconstriction due to an overactivity of vasoconstrictor fibres to capacitance vessels, or both. Simon (1976) showed that the venous pressure-volume curves are shifted to the pressure axis in SHR compared to normotensive control rats indicating a decreased venous capacity in SHR. Also, Olsson and Nilsson (personal communication) noted a reduced distensibility of SHR capacitance vessels compared with normotensive controls. At complete relaxation of the vascular bed, however, the resting situation SHR shows a considerable elevation of left atrial pressure (Norell et al. 1979). A decreased distensibility of the left atrial end of the pulmonary capacitance system are most likely to be secondary to the increased pressure in the left atrium and the pulmonary veins. Greenberg et al. (1978) found that the portal venous and caval vein and the pulmonary artery exhibit some wall hypertrophy in SHR. Whatever the mechanism behind the decreased distensibility of systemic and pulmonary veins in SHR, a given volume

load will be more centralized in SHR than in Wistar-Kyoto rats (Wistar). This will in turn lead to a greater distension of left atrial walls in SHR, to a greater receptor activation and consequently to an accentuated reflex sympathetic inhibition. This, in spite of the assumed decreased distensibility of the left atrial wall in the SHR, which per se should counteract this accentuated reflex response to a volume load. Still, this hypothesis has to be proven.

Physiological significance of augmented volume efflux in SHR

A surprising finding in the present study was that mean arterial pressure increased less in SHR than in Wistar-Kyoto rats upon 10% blood volume expansion. Thus, the augmented volume receptor reflex response in SHR allows these animals to better cope with increases in blood volume in at least terms of increases in arterial pressure and therefore with less of arterial baroreceptor activation.

Another interesting phenomenon in hypertensive animals and man is the exaggerated natriuretic response to saline load (Wilks et al. 1976). It is possible that this is at least partly due to such an accentuated reflex sympathetic inhibition of low-pressure receptors in SHR upon volume loading, because renal sympathetic nerves are known to markedly influence both sodium excretion (DiBona 1977) and renin release (Zanchetti 1976).

REFERENCES

- BROWN A.M., SAUM W.R. & TULEY F.H. 1976 A comparison of aortic baroreceptor discharge in normotensive and spontaneously hypertensive rats. *Circ Res* 39, 688-696.
- DiBONA, G.E. 1977 Neurogenic regulation of renal tubular sodium excretion. *Amer J Physiol* 233, F73-F81.
- LINDEN R.J. 1976. Reflex from receptor in the heart. *Cardiology* 41, Suppl. 1, 7-30.
- NIKOLAEVIC, B., DOLGORA, KORUBIN V. & TADZIER, I.S. 1972 Blood volume and spontaneously hypertensive rats. *Acta Med Jugoslav* 26, 251-256.
- NORELL, E., RICKSTEN S.E. & THOREN P. 1979 Left atrial pressure in normotensive and spontaneously hypertensive rats. *Acta Physiol Scand* 107, 9-12.
- RICKSTEN S.E., NORELL E. & THOREN P. 1979 Inhibition of renal sympathetic nerve traffic from cardiac receptors in normotensive and spontaneously hypertensive rats. *Acta Physiol Scand* 106-107, 17-22.
- RUPPE, W., LUNDIN S. & FOLKOW B. 1978 Plasma volume, blood volume and transcapillary escape rate (TER) of albumin in young spontaneously hypertensive

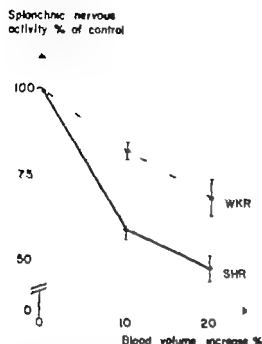


Fig. 5 The estimated contribution of cardiac receptors to reflex sympathetic inhibition in response to volume load. The curve is derived from a subtraction of the values in Fig. 4 from the corresponding values in Fig. 3.

Fig. 5 shows the estimated reflex sympathetic inhibition evoked from cardiopulmonary receptors. This figure is derived from the difference between total sympathetic inhibition (Fig. 3) and that caused by arterial baroreceptors (Fig. 4). Fig. 5 shows that a 10% and 20% volume load activates cardiopulmonary receptors more in SHR leading to an exaggerated reflex inhibition of splanchnic nerve activity in hypertensive animals compared to normotensive ones.

DISCUSSION

The most significant finding in this study is the fact that SHR shows a more pronounced reflex inhibition (Fig. 2) of splanchnic sympathetic activity upon volume load than normotensive controls. Thus at a 10% increase of blood volume sympathetic discharge decreased significantly more in SHR than in WKR. We here assume that the total blood volume per 100 g b.w. is about the same in SHR and WKR as reported by Rippe et al. (1978). Other investigators report a 17.5% greater blood volume in SHR compared to normotensive Wistar rats (Nikodyjevic et al. 1977). If this is true the reflex inhibitory response to a given volume load (ml/100 g) would be even more marked than shown in Fig.

2. In a recent study it is shown that the total volume is 10% greater in WKR compared to an age of 3-4 months (Lundin personal communication). If Fig. 2 is recalculated according to Lundin's data there is still a significant reflex sympathetic inhibition in SHR than in WKR at a volume expansion of 10% ($P < 0.01$).

Arterial baroreceptor contribution. What is the reason for this more powerful reflex inhibition response to volume load in SHR? It is not mediated via arterial baroreceptors because if any difference in sensitivity of the baroreceptor reflex exists between SHR and WKR, it is that SHR tends to inhibit sympathetic discharge less than WKR, though this difference is not statistically significant. Such reduced sensitivity of the arterial baroreceptors in SHR has been reported by several previous investigators (Saw Wang 1976; Brown et al. 1976).

In addition, arterial pressure increased less in SHR upon volume load (Fig. 3). Fig. 4 shows the estimated contribution of arterial baroreceptors to reflex sympathetic inhibition upon volume load in SHR and WKR. These receptors seem to contribute much less in SHR than in WKR, but the sensitivity of the baroreceptor reflex may be decreased in SHR and furthermore the lower arterial pressure was smaller in SHR. We are aware that objections can be raised against such an interpretation as shown in Fig. 4 because the baroreceptor sensitivity is obtained by noradrenaline infusion and this value for baroreceptor sensitivity is used to estimate the baroreceptor contribution to the reflex sympathetic inhibition upon volume load. In these two situations the pulse-pressure is likely to be different also at similar changes in mean arterial pressure. However, the markedly decreased arterial baroreceptor contribution to reflex sympathetic inhibition in response to volume load in SHR is highly likely to be real even when taking this objection into consideration.

The role of cardiopulmonary receptors. What is the reason for the augmented reflex response to volume load in SHR? A priori the most likely explanation is an augmented low pressure receptor activation. Thus there exist throughout the cardiopulmonary area a large population of endings with nonmedullated vagal afferents which have tonic nervous inhibitory influence on cardiovascular centers (Thoren 1979). The endings respond to elevations of central blood pressure and

Temporal dissociation between the negative inotropism and the increase in cyclic GMP level induced by acetylcholine esters in spontaneously beating rat atria preparations¹

METSÄ-KETELÄ, R. KUOSA and H. VAPAATALO
Department of Biomedical Sciences, University of Tampere, Finland

METSÄ-KETELÄ T, KUOSA R. & VAPAATALO H. Temporal dissociation between the negative inotropism and the increase in cyclic GMP level induced by choline esters in spontaneously beating rat atria preparations. *Acta Physiol Scand* 1980; 110: 83-87. Received 20 Dec. 1979. ISSN 0001-6772. Department of Biomedical Sciences, University of Tampere, Finland.

The effects of acetylcholine and carbachol on cyclic GMP levels and contraction amplitude were investigated simultaneously in spontaneously beating rat atria preparations. Both drugs at the concentrations of $5 \cdot 10^{-6}$ M or higher produced a very rapid decline of amplitude. However, the cyclic GMP level was not elevated earlier than 5 s later when the amplitude was already reduced to 50% of the initial value. Concentrations of acetylcholine below $5 \cdot 10^{-6}$ M were not able to affect the cyclic GMP level, although they reduced the contractility significantly. Acetylcholine had no effect on the cyclic AMP level. The data demonstrate that cyclic GMP does not mediate the negative inotropic effect of choline esters in rat atria preparations.

Key words: Acetylcholine, cyclic GMP, negative inotropism, heart.

In 1970, when George et al (1970) suggested a possible relationship between guanosine 3',5'-cyclic phosphate (cyclic GMP) and the contraction rates of the heart, it has been concluded in several papers that there is a correlation between negative inotropism and an increase in cyclic GMP level in the heart (Lee et al 1972, George et al 1973, George et al 1975, Watanabe & Besh 1975, Fink et al 1976) after acetylcholine (ACh) administration. Temporal relationship between the negative inotropism and the increase of cyclic GMP after ACh has been reported (George et al 1970, George et al 1973, George et al 1975, Fink et al 1976). In favour of the hypothesis on cyclic GMP-mediated negative inotropism.

This hypothesis is contradicted by the observations that low concentrations of choline esters are unable to increase the cyclic GMP content, although they reduce cardiac contractility (Diamond et al 1977, Brooker 1977). Some drugs, e.g. sodium tropinide, also increase markedly the cyclic AMP level without any effect on contractility (Diamond et al 1977, Katsulis et al 1977, Keely & Lincoln 1978). It has also been reported that ACh

induces various actions on the heart including increase in cyclic GMP, but the actions are only partially mediated by cyclic GMP (Nawroth 1977).

To study the role of cyclic GMP in the negative inotropic effect of ACh, we have used spontaneously beating rat atria preparations, which can be frozen very rapidly and a 5 s timing scale in sampling during the initial period of the negative inotropism.

MATERIALS AND METHODS

Isolated spontaneously beating rat atria preparation. We used Fed male Wistar rats (weight 190-220 g) were killed by dislocation of the neck. The hearts were immediately excised, immersed and washed free from blood in Tyrode solution containing 1000 IU/l heparin at room temperature. The Tyrode content (g/l) was: NaCl 8.0, KCl 0.2, CaCl₂ 2.0, H₂O 0.18, MgCl₂ 6.0, NaH₂PO₄ 2.0, H₂O 0.05, HCO₃⁻ 0.4, glucose 1.0. The pH was 7.4. The atria were separated from ventricles in heparinized Tyrode bubbled continuously with pure

The results have been partly presented in Vth International Symposium on Medicinal Chemistry, Brighton 4-7 September 1978.

- sive rats (SHR) as compared with normotensive controls (NCR) *Clin exp hypertension* 1: 39-51
- SAPRU H N & WANG S C 1976. Modification of aortic baroreceptor resetting in spontaneously hypertensive rat. *Amer J Physiol* 230: 664-674
- SIMON G 1976. Altered venous function in hypertensive rats. *Circ Res* 38: 412-418
- THORÉN P 1979. Role of cardiac vagal C-fibres in cardiovascular control. *Rev Physiol Biochem Pharmacol* (Springer Verlag) 86: 1-94
- THORÉN P NORESSON E & RICKSTEN S E 1979. Cardiac receptors with vagal afferents in the rat. *Acta Physiol Scand* 105: 294-303
- THORÉN P NORESSON E & RICKSTEN S E 1979. Resetting of cardiac C-fiber endorgans in spontaneously hypertensive rat. *Acta Physiol* 107: 13-18
- WILLIS L R McCALLUM P W & HIGGINS B 1976. Exaggerated natriureses in the spontaneously hypertensive rat. *J Lab Clin Med* 77: 777
- ZANCHETTI A STELLA A LEONETTI G MORGANTI A & TERYOLI L 1976. Correnin release: A review of experimental evidence and clinical implications. *Amer J Cardiol* 37: 674-685

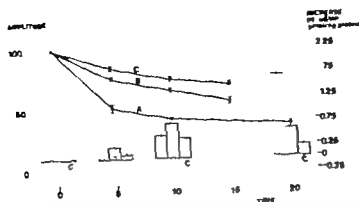


Fig. 3. Effects of various concentrations of acetylcholine on the amplitude and cyclic GMP levels of spontaneously beating rat atria preparations. The final concentrations of acetylcholine in the incubation medium were $1 \cdot 10^{-6}$ M (A), $5 \cdot 10^{-6}$ M (B) and $1 \cdot 10^{-5}$ M (C). Each point represents the mean \pm S.E. of the amplitude and the columns represent \pm S.E. of the change in cyclic GMP level. S.E. and statistical significance ($P < 0.05$) has been calculated from the original data.

Tris HCl buffer, pH 7.6 (Buffer 1). The assay was carried out as follows: 50 μ l of sample or standard (10^{-4} to 10^{-10} mol/l) in Buffer 1, 25 μ l [3 H]-cyclic GMP (0.5 μ l in Buffer 1 containing 100 mM MgSO_4). The incubation at 37°C was initiated by adding 25 μ l of cyclic AMP dependent protein kinase in 5 mM potassium phosphate buffer, pH 7.0, and stopped by 1 ml of 0°C potassium phosphate buffer (20 mM, pH 6.0). One ml of this solution was filtered through a mixed ester cellulose filter (Mallinckrodt, GSWP, 0.22 μm). The disc was dried at $60-70^\circ\text{C}$ for 20 min and dissolved in 500 μ l of dimethylformamide. The scintillation cocktail (Liposol, Lucina AG Basel, Switzerland) was added and the radioactivity was measured by a scintillation counter.

In both assays, the zero binding level was kept between 10 and 25%. The pellet of trichloroacetic acid extract was dissolved in 1 ml 1 M NaOH and the protein content measured by the method of Lowry et al. (1951).

To measure the recovery 125 μ l of the resuspended atria was transferred into scintillation vial and 4.5 ml aqueous based scintillation solution (Aquafluor, Lucina AG Basel, Switzerland) was added. From the radioactivity the recovery of the extraction was calculated and taken consideration in the final concentrations. The recovery of the extraction of cyclic AMP varied from 90 to 100%. The fraction used for cyclic GMP assay contained 75% of [3 H]-cyclic GMP added.

Chemicals: Acetylcholine chloride (Sigma Chemicals, St. Louis, Mo, USA), cyclic AMP and cyclic GMP (Boehringer Mannheim GmbH, Mannheim, Germany), [3 H]-cyclic AMP and [3 H]-cyclic GMP (Radiochemical Centre Ltd, Amersham, England) and the cation exchange resin AG50W X4 (200-400 mesh, hydrogen form, Bio-Rad Laboratories, Richmond, Calif., USA).

For testing the statistical significance, Student's independent t-test was used.

RESULTS

The time courses of the effects of acetylcholine on contractility and cyclic GMP are presented in Fig. 1. The negative inotropic effect appears immediately after the administration of ACh ($1 \cdot 10^{-6}$ M) and reaches the maximum at 20 s. The initial decline of the amplitude is very rapid, and the 50% level of the initial value is reached in 5 s. There is at least a 5 s delay in the onset of the increase in cyclic GMP level, which is elevated in 10 s ($P < 0.05$). The maximum is reached in 30 s ($P < 0.01$). The cyclic AMP level remains unaltered during the testing period. Carbachol (CCh), similarly to ACh, changes the contractility and cyclic GMP level (Fig. 2). Atropine ($1 \cdot 4 \cdot 10^{-6}$ M) abolishes both the negative inotropic effect and the cyclic GMP elevation by ACh ($1 \cdot 10^{-6}$ M).

The dose-response curves of ACh on the increase in cyclic GMP and in amplitude are presented in Fig. 3. The threshold concentration of ACh to produce an increase in cyclic GMP level in rat atria preparation is $5 \cdot 10^{-7}$ M. Concentrations below this are however able to depress the contractility.

DISCUSSION

Our results do not support the hypothesis suggested by George et al. (1970) that cyclic GMP could mediate the negative inotropic effect of acetylcholine. In our preparation, ACh in concentrations

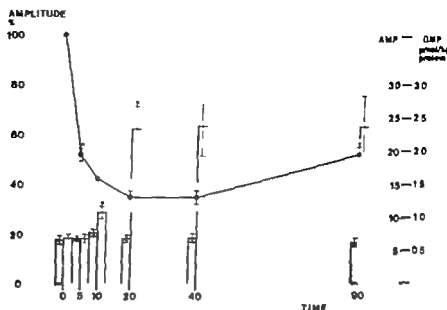


Fig. 1 Effects of acetylcholine on the amplitude (●—●) on the cyclic GMP (open column) and the cyclic AMP (shaded columns) levels of spontaneously beating rat atria preparations. The atria were preincubated for 45 min in Tyrode solution and then to the final concentration of 1×10^{-6} M acetylcholine. Each point or column represents the mean \pm S.E. of 8 atria. Asterisk indicates a statistically significant change ($P < 0.05$) from the control value.

oxygen. A polyester suture was tied to the apex of both atria and they were transferred to an organ bath (70 ml $+30^\circ\text{C}$) with continuous flow (1.3 ml/min) and bubbling of pure oxygen. The oxygen saturation was monitored with a Clark's electrode (Yal Model 53 Oxygen Monitor, Yellow Springs Instrument Co., Yellow Springs, Ohio, USA) and kept at the maximum (about 95% saturation). Contractions of the atria were registered isometrically by Harvard Apparatus Isometric Force Transducer model 363 and Amplifier model 390. Four preparations were stabilized at the same time after 45 min a drug was given in a 200 μl volume and the atria were frozen by Wollenberger clamps precooled in liquid nitrogen. The fixation procedure from cutting of suture to frozen tissue took 1 min. The contractility, adenosine 3',5'-monophosphate (cyclic AMP) and cyclic GMP were measured from the same preparations. The atria were stored for the analysis at -70°C .

Cyclic GMP and cyclic AMP assays. Tissues were pulverized by a microsmembrator (B. Braun, Melsungen, Germany) in a teflon vial precooled in liquid nitrogen with a 13 g steel ball. The pulverized atria (100–150 mg) were put into 1.5 ml polyethylene vials (Eppendorf GmbH, Eppendorf, Germany). The 500 μl of 5% trichloroacetic acid was added and the suspension was heated at 95°C for 2 min. The extraction of the nucleotides was promoted by vigorous shaking for 15 min at room temperature. After centrifugation (9000 \times g, 15 min) the supernatant was divided into two portions: 400 μl for cyclic GMP and 40 μl for cyclic AMP assays. To the cyclic AMP ml 40 μl of H_2O was added for monitoring the recovery: about 85 Bq of the corresponding tritiated cyclic nucleotide ($50 \mu\text{l}$ of H_2O was added to each sample). Water saturated diethyl ether washing was repeated three times after lowering the

pH 5–3.0 by 10 μl of 0.1 M HCl. The cyclic AMP lyophilized and assayed without any further purification by the method of Gilman (1970).

The cyclic GMP in the 400 μl of ether washed supernatant was separated from the disturbing cyclic AMP by 50V–4 chromatography in a 9×45 mm column. It eluted with H_2O and a fraction from 800 to 1800 μl collected. Cyclic AMP was eluted after 1800 μl fraction collected was lyophilized. Cyclic GMP measured by the competitive protein binding assay (Dinnendahl, 1974) using partially purified bovine muscle cyclic GMP-dependent protein kinase. Lyophilized cyclic GMP was resuspended in 50 μl of

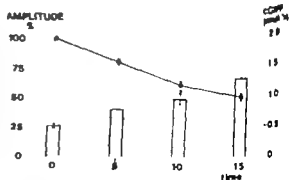


Fig. 2 Effect of carbacholine (10^{-6} M) on the amplitude (●—●) and on the cyclic GMP level (column). Each point or column represents the mean \pm S.E. of atria preparation ($P < 0.05$).

- JKER, G. 1977. Dissociation of cyclic GMP from the parasympathetic action of carbachol in guinea pig rat. *J Cyclic Nucleot Res* 3: 407-413.
- JOND, J., TENEICK, R. E. & TRAPANI, A. J. 1977. Are increases in cyclic GMP levels responsible for the vagal inotropic effects of acetylcholine in the heart. *Biochem Biophys Res Commun* 79: 91-918.
- KENDALL, V. 1974. A rapid and simple procedure for the determination of guanosine 3',5'-monophosphate by use of the protein-binding method. *Naturwissenschaften* 28: 55-61.
- KOHN, P., PADDOCK, R. J., ROGERS, G. H., LUTILL, R. W. & GEORGE, W. J. 1976. Elevated cyclic GMP levels in rabbit atria following vagal stimulation and acetylcholine treatment. *Proc Soc Exp Biol Med* 153: 78-82.
- KOHN, W. J., WILKERSON, R. H. & KADOWITZ, P. J. 1973. Influence of acetylcholine on contractile force and cyclic nucleotide levels in the isolated perfused rat heart. *J Pharmacol Exp Ther* 184: 223-235.
- KOHN, W. J., POLSON, J. H., OTOOLE, A. G. O. & GOLDBERG, N. O. 1970. Elevation of guanosine 3',5'-cyclic phosphate in rat heart after perfusion with acetylcholine. *Proc Natl Acad Sci U.S.A.* 66: 398-403.
- KOHN, W. J., IGARRRO, L. J., PADDOCK, R. J., WHITEL, L. A. & KADOWITZ, P. J. 1973. Oppositional effects of acetylcholine and isoproterenol on somatic tension and cyclic nucleotide concentrations in rabbit atria. *J Cyclic Nucleot Res* 1: 339-347.
- MAN, A. G. 1970. A protein binding assay for adenosine 3',5'-cyclic monophosphate. *Proc Natl Acad Sci U.S.A.* 67: 305-312.
- MATTHEI, O. F. & TRAUTWEIN, W. 1966. Vagal and sympathetic effects on the pacemaker fibres in the sinus venosus of the heart. *J Gen Physiol* 39: 715-733.
- KATSUKI, S., ARNOLD, W. P. & MURAD, F. 1977. Effects of sodium nitroprusside, nitroglycerin and sodium azide on levels of cyclic nucleotides and mechanical activity of smooth tissues. *J Cyclic Nucleot Res* 3: 239-247.
- KEELY, S. L. Jr & LINCOLN, T. H. 1978. On the question of cyclic GMP as the mediator of the effect of acetylcholine on the heart. *Biochem Biophys Acta* 543: 251-257.
- LEE, T. P., KUO, J. F. & GREENGARD, P. 1977. Role of muscarinic cholinergic receptors in regulation of guanosine 3',5'-cyclic monophosphate content in mammalian brain, heart muscle and intestinal smooth muscle. *Proc Natl Acad Sci U.S.A.* 69: 3287-3291.
- LINCOLN, T. H. & CORBIN, J. D. 1977. Adenosine 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate-dependent protein kinases. Possible homogenous proteins. *Proc Natl Acad Sci U.S.A.* 74: 3239-3243.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. 1951. Protein measurements with the folin phenol reagent. *J Biol Chem* 193: 265-275.
- MITTAL, C. E. & MURAD, F. 1977. Properties and oxidative regulation of guanylate cyclase. *J Cyclic Nucleot Res* 3: 381-391.
- NAWRATH, H. 1977. Does cyclic GMP mediate the negative inotropic effect of acetylcholine in the heart? *Nature (Lond.)* 267: 72-74.
- THELSON, M., FRIEDRICH, M., JUSTUS, P., GÜTTLER, K. & KLAUS, W. 1977. Heat production and oxygen consumption of the isolated rabbit heart: their relations to mechanical function. *Basic Res Cardiol* 72: 19-33.
- WATANABE, A. M. & BESH, H. R. J. 1975. Interaction between cyclic adenosine monophosphate and cyclic guanosine monophosphate in guinea pig ventricular myocardium. *Circulat Res* 37: 309-317.

of 5×10^{-7} M or higher reduced at first the amplitude and a few seconds thereafter the level of cyclic GMP increased. Low concentrations did not change significantly the cyclic GMP level in spite of decreased contractility.

The time response curves of ACh for negative inotropic effect and for increase in cyclic GMP have been presented earlier (George et al 1970; George et al 1973; George et al 1975; Fink et al 1976) with the conclusion that cyclic GMP may mediate the negative inotropic effect of ACh. The preparations used in those studies were isolated perfused rat hearts (George et al 1970; George et al 1973) and isolated paced rabbit atria (George et al 1975; Fink et al 1976). The negative inotropic response to ACh developed very slowly compared to spontaneously beating rat atria in the present study. The contractility of our preparation was reduced on the same time scale as in the rabbit atria preparation in situ after stimulation of the vagus nerve (Fink et al 1976). In the paced rabbit atria preparation (George et al 1975) there seems to be no established time difference in the mechanical responses of the vagal and sympathetic nerves (Hutter & Trautwein 1956) on the heart. In the preparation used in the present study the difference is more than 5 s (data not present).

Recently Keely & Lincoln (1978) have reported that both ACh and sodium nitroprusside can increase the cyclic GMP level of perfused rat heart but only ACh is able to depress the contractility. From the figures of that study even the existence of a temporal difference in those two responses of ACh can be assumed.

Low concentrations of ACh (5×10^{-7} M) have been reported to produce a decrease in the contractility of cat atrial strips (Diamond et al 1977) without any change in cyclic GMP. This agrees with the present study in which the threshold concentration for the cyclic GMP elevation is 5×10^{-7} M. CCh up to 1×10^{-6} M concentration has also been reported to be ineffective in altering the cyclic GMP level but to be able to depress very effectively the tension developed (Brooker 1977).

The cardiac contractility correlates to the oxygen consumption of the heart very well (Braunwald 1971; Theisohn et al 1977). ACh in a concentration of 1×10^{-6} M in the present study produced a very rapid decline of the contractility which could thus be expected to affect heavily the oxygen consumption and thereby the redox state. The redox state

and formation of free radicals have been reported to be the most important physiological determinants of the activation of the guanylate cyclase (N. Murad 1977).

It may be possible that ACh either directly reducing the contractility first disturbs the balance between supply and demand of oxygen and then the redox state which then activates the guanylate cyclase. The observation that low concentrations of ACh do not affect the cyclic GMP level indicate that the initial decline of contractility is quite severe to divert the redox state enough to guanylate cyclase activation process.

We suggest that cyclic GMP cannot be mediator of the contractility changes in the reduced negative inotropism. Because the increase in cyclic GMP is temporally closely associated but not preceded by a decline of the increased heart rate and contractility it must have some functions in this process. Cyclic GMP activates 6-phosphofructokinase (EC 2.7.1.11) (Beitner 1977). This effect is most pronounced when the enzyme is already partially inhibited by ATP or citrate, e.g. under conditions in which the situation is good. Cyclic GMP also abolishes antagonistic action of cyclic AMP and other cAMP activators such as glucose 1,6-bisphosphate or AMP on the ATP or citrate inhibited (Beitner et al 1977). In addition, bovine lung cyclic GMP dependent protein kinase has been shown to phosphorylate many glycogen degrading and relaxing enzymes (Lincoln & Corbin 1977). This suggests that cyclic GMP in addition to the effect of the reduced cytoplasmic calcium concentration can mediate the metabolic effects of ACh and can reduce the energy production to a lower level corresponding the reduced energy demand.

This research was supported by a grant from The Finnish Medical Research Foundation, Finland. We are grateful to Mrs Siikka Heikin and Mrs Yappo Halla for Skilful technical assistance.

REFERENCES

- BEITNER E, HABERMAN S & CYCOMITZ J 1977 The effect of cyclic GMP on phosphofructokinase from rat tissues. *Biochim Biophys Acta* 330: 140.
- BRAUNWALD E 1971 Control of myocardial oxygen consumption: physiologic and clinical considerations. *Amer J Cardiol* 27: 416-43.

Effects of nine different gastrointestinal polypeptides on vascular smooth muscle in vitro

HELLSTRAND and JOHANNES JÄRHULT

Department of Physiology and Biophysics, University of Lund, Sweden

HELLSTRAND P & JÄRHULT J. Effects of 9 different gastrointestinal polypeptides on vascular smooth muscle in vitro. *Acta Physiol Scand* 1980; 110: 89-94. Received 27 Dec. 1979. ISSN 0001-6772. Department of Physiology and Biophysics, University of Lund, Sweden.

Nine polypeptides of gastrointestinal origin were tested for their possible effect on vascular smooth muscle of the rat portal vein. The substances tested were bombesin, carbachol, glucagon, insulin, pentagastrin, secretin, somatostatin, substance P and vasoactive intestinal polypeptide (VIP). Cumulative dose-response relations of integrated mechanical activity (area tension) were obtained at basal concentrations of the various peptides of 1-10 µg/ml. Within this concentration range only substance P and VIP showed clearcut effects; substance P causing contraction and VIP relaxation. The dose of substance P needed to produce contraction was high ($ED_{50} > 1 \mu M$) so that the physiological importance of this response is doubtful. On the other hand, ED_{50} for the relaxing effect of VIP was about 15 nM, which is in accordance with concentrations reported to produce significant vasodilatation *in vivo*. The results support the view that vascular effects which have been reported to occur as response to the other 7 peptides are mainly of indirect origin and not mediated via direct action on vascular smooth muscle.

Key words: vascular smooth muscle, polypeptides, bombesin, carbachol, glucagon, insulin, pentagastrin, secretin, somatostatin, substance P, VIP.

Several of the gastrointestinal polypeptides have ascribed effects on blood flow through many different vascular beds (e.g. Thulin 1973; Bertaccini 1976). However, in many of these previous studies peptides have been infused in large doses and the effects on blood flow distribution have been rather inconsiderable. These findings suggest that blood flow responses represent pharmacological rather than physiological effects or that the peptides act on the blood vessels via indirect mechanisms. The vascular smooth muscle of the rat portal vein plays spontaneous myogenic activity and the critical mechanical and metabolic properties of its preparation have been characterized in detail (Ljung 1970; Hellstrand 1979). In the present investigation we have used the portal vein to test the different gastrointestinal polypeptides with regard to their possible vasoactive properties. The study has shown that only two of them are potent vasoactive substances *in vitro*, viz. VIP and substance P.

METHODS

Portal veins were dissected from male Sprague-Dawley rats and suspended in organ baths made from glass syringes (plunger removed) mounted vertically in stirred water at 37°C. Two or four branches were used simultaneously, each mounted in separate syringe. The muscles were connected to Grass FTO3 force transducers and kept at preload of 5 mN. The syringes were filled with 4 ml of Krebs solution of the following composition (mM): NaCl 122, KCl 4.73, $CaCl_2$ 2.50, $MgCl_2$ 1.19, $NaHCO_3$ 15.5, KH_2PO_4 1.19, glucose 11.5, $CaNa_2$ -versenate 0.026. The solution was bubbled with a mixture of 95% O_2 and 4% CO_2 , giving pH of 7.3-7.4.

After an equilibration period of about 45 min, the experiments were performed according to the following protocol. First, spontaneous activity of the portal vein was recorded for 10 min (=control period). The peptide to be tested, dissolved in 0.2 ml Krebs solution, was then added cumulatively in succeeding doses. Each dose was allowed to act for 10 min and the volume in the bath was adjusted to 4 ml before addition of the next dose. In most expts. 6 doses were given, differing each by a factor of 5. After the response to the highest dose had been recorded, the bath was raised twice and the muscle was left to recover for

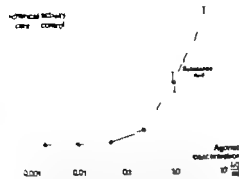


Fig. 5. Dose-response relation showing integrated activity of portal vein on exposure to substance P in 4 expts. Activity 15 sec after washout was $88 \pm 12\%$.

This strong excitatory response to substance P is fully reversible.

active site: natural polypeptide (VIP)

Fig. 3 illustrates original recordings of spontaneous activity of a portal vein in response to three different doses of VIP. This peptide caused a marked cessation of the amplitude of spontaneous contractions while the frequency of contractions was little altered or somewhat increased.

Fig. 4 sets out the compiled results from 4 expts which the effects of VIP on mechanical activity were tested. The two records marked were obtained with freshly dissolved VIP whereas records were obtained with peptide that had been frozen a few hours (see Methods). With freshly dissolved VIP a dose of $1 \mu\text{g/ml}$ invariably caused complete cessation of spontaneous activity. The effect of high doses of VIP were not completely reversible since activity after washout of the pep-

tide was only about 60% of that obtained during the initial control period.

Somatostatin

Fig. 5 shows that somatostatin caused a slight depression of mechanical activity to about 75% of the control value at the highest dose. The recovery control activity was only 90% of the initial activity so it is possible that the apparent depression of activity by somatostatin is in part due to fatigue.

Bombesin, caerulein, glucagon, insulin, pentagastrin and secretin

Figs. 6 and 7 show that none of the 6 other peptides tested had any significant effect on the mechanical activity of the rat portal vein. At high doses caerulein and secretin caused a slight excitation, whereas bombesin, pentagastrin and insulin caused some inhibition. The response of the muscles to glucagon was indifferent. Except after caerulein and secretin, the control activity at the end of each expt was reduced to 55–75% of the initial value indicating that some of the peptide-induced inhibition of activity was not reversible and/or that some of it was due to fatigue.

DISCUSSION

Of the 9 polypeptides tested in this study only substance P and VIP had a marked effect on the mechanical activity of the rat portal vein. The constrictory action of substance P noted here is in accordance with reported effects on intestinal and respiratory smooth muscle *in vitro* (Euler & Gaddum 1931; Pernow 1960; Nilsson et al. 1977; Cocks & Burnstock 1979), but contrasts to its vasodilatory

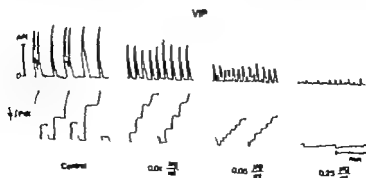


Fig. 3. Effect of VIP on mechanical activity of portal vein. Records taken as in Fig. 1.

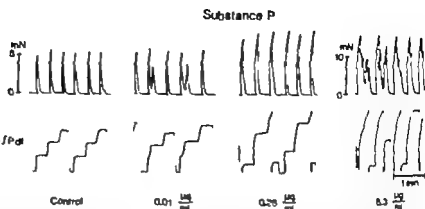


Fig. 1 Effect of substance P on mechanical activity of the rat portal vein. Records from last 2 min of 10 min exposures to the concentrations indicated. Upper records, active isometric tension. Lower records, integrated active tension over 1 mm intervals, in arbitrary units. Preload 5 mN.

15 min. Recovery control activity was then recorded during 5 min.

In all expts the mechanical activity of the portal vein was recorded with electronic integration of the tension in 1 min periods. The mean output from the integrator during the whole initial period was used as the control value of mechanical activity, whereas the mean integrated activity in response to the actual peptide was calculated from the values obtained during the final 5 min of each "peptide" period. Dose response curves were then constructed by setting the control activity of each muscle at 100%.

All peptides were kept frozen, except for insulin which was refrigerated. When available as pure substance the peptides were dissolved in saline either freshly on the day of the expt or prepared as stock solutions which were immediately frozen. On one occasion a peptide (VIP) had to be frozen after an expt and used again the following day, which resulted in a marked loss of activity (see Fig. 4).

The following peptides were used: Bombesin (Bachem) caerulein (Farnitalia), glucagon (Novo), insulin (Vitrum), pentagastrin-1CT, secretin (GIH Research Unit, Karolinska Institutet, Stockholm), somatostatin (Serono), substance P (Beckman) and VIP (generously supplied by Prof. V. Mutt, GIH Research Unit, Karolinska Institutet, Stockholm).

Doses given are expressed as $\mu\text{g/ml}$ final concentration except in the case of insulin, where the concentrations are expressed as IU/ml. For secretin, conversion factor of 3.4 clinical units per μg was used (Vagne et al. 1968).

Results are given as mean values \pm S.E.

RESULTS

Of all the substances tested for their effect on the rat portal vein (bombesin, caerulein, glucagon, insulin, pentagastrin, secretin, somatostatin, substance P, and vasoactive intestinal polypeptide (VIP)) only substance P and VIP had prominent effects on isometric tension. Dose-response relations for each

peptide were obtained with activity of the portal veins quantitated as integrated (mean) tension. To test for possible effects of fatigue, Krebs solution was administered to control muscles according to the same protocol as the expts with active peptides. At the time of addition of the sixth dose, activity was $90 \pm 7\%$ of initial value, indicating that a reduction in activity of about 10% may be expected on the basis of fatigue alone.

Substance P

Fig. 1 shows representative recordings of the effects of 3 different concentrations of substance P on isometric tension of the portal vein. Below the tension records is shown the output from the electronic integrator giving the time integral of tension in 1 min periods. The recordings show the median activity during the last min of the 10 min period, that each dose was allowed to act on the preparation. As can be seen, substance P contracted the portal vein at all doses tested. However, even at the highest dose ($6.3 \mu\text{g/ml}$) a fully fused tetanus response such as occurs e.g. with high doses of adrenaline did not appear. The excitatory effect of substance P on the portal vein was not affected by phenoxybenzamine ($1 \mu\text{M}$) or atropine ($10 \mu\text{M}$). Generally, full effect was seen in about 1 min after addition of the peptide and the effect was well maintained during the whole 10 min period.

Fig. 2 summarizes the results from 4 expts with substance P. As can be seen, substance P at doses exceeding $0.1 \mu\text{g/ml}$ caused marked increases in mechanical activity. At the highest dose ($6.3 \mu\text{g/ml}$) mechanical activity was about 70% of the control

chemical activity
control

Substance P

Agonist concentration
10 μM

Dose-response relation showing integrated activity of portal vein on exposure to substance P in 4 experiments. Activity 15 min after washout was $88 \pm 12\%$

This strong excitatory response to substance P is fully reversible.

Vasoactive intestinal polypeptide (VIP)

Fig. 3 illustrates original recordings of spontaneous activity of a portal vein in response to three different doses of VIP. This peptide caused a marked reduction of the amplitude of spontaneous contractions while the frequency of contractions was little affected, or somewhat increased.

Fig. 4 sets out the compiled results from 4 experiments which the effects of VIP on mechanical activity were tested. The two records marked * were obtained with freshly dissolved VIP whereas records 1 and 2 are obtained with peptide that had been frozen for a few hours (see Methods). With freshly dissolved VIP a dose of $1 \mu\text{g/ml}$ invariably caused complete cessation of spontaneous activity. The effect of high doses of VIP were not completely reversible since activity after washout of the pep-

tide was only about 60% of that obtained during the initial control period.

Somatostatin

Fig. 5 shows that somatostatin caused a slight depression of mechanical activity to about 75% of the control value at the highest dose. The recovery of control activity was only 90% of the initial activity so it is possible that the apparent depression of activity by somatostatin is in part due to fatigue.

Bombesin, arginine glycine isoleucine pentagastrin and secretin

Figs. 6 and 7 show that none of the 6 other peptides tested had any significant effect on the mechanical activity of the rat portal vein. At high doses, caerulein and secretin caused a slight excitation, whereas bombesin, pentagastrin and insulin caused some inhibition. The response of the muscles to glucagon was indifferent. Except after caerulein and secretin, the control activity at the end of each experiment was reduced to 55–75% of the initial value indicating that some of the peptide-induced inhibition of activity was not reversible and/or that some of it was due to fatigue.

DISCUSSION

Of the 9 polypeptides tested in this study only substance P and VIP had a marked effect on the mechanical activity of the rat portal vein. The constancy of action of substance P noted here is in accordance with reported effects on intestinal and respiratory smooth muscle *in vitro* (Euler & Gaddum 1931; Pernow 1960; Nilsson et al. 1977; Cocks & Burnstock 1979), but contrasts to its vasodilatory

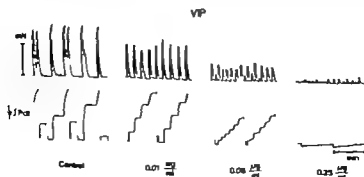


Fig. 3 Effect of VIP on mechanical activity of portal vein. Records taken as in Fig. 1

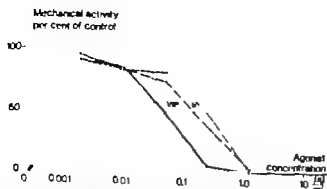


Fig. 4. Dose-related effect of VIP on portal veins. Data from 4 individual vessels shown. Records marked *a* show results with freshly dissolved VIP whereas records *b* were obtained using VIP which had been frozen after a few hours. Mean recovery activity was $62 \pm 8\%$.

action *in vivo* (Euler & Gaddum 1931; Löfström et al 1965; Hallberg & Pernow 1975; Pernow & Rosell 1975; Kaneto et al 1978). However, no dilatory action of substance P on isolated vascular smooth muscle has been reported so far. In the rabbit aorta it produces contraction (Moore 1979) and we have got unpublished data to demonstrate that it contracts the rat aorta as well. Thus, the constrictory action of substance P on the rat portal vein is not an exceptional response. Certainly, the concentrations of substance P needed to produce contraction *in vitro* (Fig. 2) are much larger than those producing vasodilatation *in vivo* (e.g. Hallberg & Pernow 1975; Kaneto et al 1978). No "maximal" response to substance P was obtained with the concentrations used here (Fig. 2) indicating that ED_{50} for its constrictory effect on the portal vein *in vitro* is well above $1.3 \mu\text{g/ml}$ ($\approx 1 \mu\text{M}$).

The relaxing effect of VIP on the spontaneous activity of the rat portal vein had an ED_{50} of about $0.05 \mu\text{g/ml}$ ($\approx 15 \text{ nM}$) see Fig. 4. This value is well in accordance with the plasma concentrations of VIP that have been shown to produce significant vasodilatation *in vivo* (e.g. Sakl & Mutt 1970; Kachelhoffer et al 1974; Eklund et al 1979; Järhult, Hellstrand & Sundler 1980) but the value is higher than the portal concentrations of VIP observed after vagal stimulation (Schaffalitzky de Muckadeil, Fahrenkrug & Holst 1977; Fahrenkrug et al 1978; Bloom & Edwards 1980). The latency in onset of the VIP effect has been a consistent observation both *in vivo* and *in vitro* (e.g. Kachelhoffer et al 1974; Cocks & Burnstock 1979) but no information is available regarding possible mechanisms underlying this phenomenon. In the study by Järhult

Hellstrand & Sundler (1980) it was shown that significantly reduced the vasoconstriction induced by sympathetic stimulation. Further work is necessary to elucidate the effects of VIP both with respect to depression of myogenic tone and to possible interaction with excitatory agents.

Several of the other peptides studied here have been proposed to influence vascular tone. For instance, somatostatin has been claimed to decrease splanchnic and hepatic blood flow in man (Wahren et al 1977; Samnegård et al 1979; Järhult et al 1979). As seen from Fig. 5 we found a slight relaxing effect of somatostatin on the portal vein but it is certainly very slight in comparison with VIP. Also, it appears as if the doses used were low compared to those required to give a possible effect on the portal vein. This would support the indirect action of somatostatin on vascular tone.

Of the remaining six peptides tested (Fig. 6) bombesin and insulin were the only ones producing any degree of relaxation of the portal vein. However, it is highly unlikely that these substances may physiologically be present in concentrations large enough to produce such effects. In fact, calcitonin is closely related to cholecystik which, like secretin and pentagastrin, produces moderate increase in intestinal blood flow (Fahrenkrug et al 1973) and interestingly in human portal blood flow (Järhult & Järhult 1979). Glucagon too has repeatedly been shown to produce vascular resistance, especially in vascular beds in the gastrointestinal tract (Scheraga et al 1959; Ross 1970; Tibblin et al 1970; McFadden & Mailman 1977) and an antagonizing effect of glucagon on the sympathetic vasoconstriction.

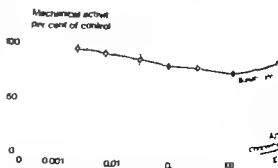


Fig. 5. Mechanical activity of portal vein on exposure to different concentrations of somatostatin. Recovery activity was $90 \pm 10\%$.

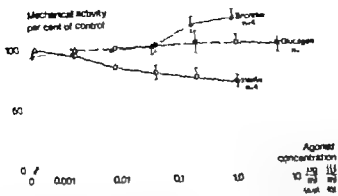


Fig. 6. Mechanical activity of portal veins on exposure to different concentrations of secretin ($\mu\text{g/ml}$), glucagon ($\mu\text{g/ml}$) and insulin (IU/ml). Recovery activities after wash-out were for secretin $90 \pm 4\%$, for glucagon $74 \pm 8\%$ and for insulin $57 \pm 6\%$.

spastic vascular bed has also been reported (rdson & Wuthrington 1977). However the t results from the portal vein show no effect cagon on this isolated vascular smooth mus- should be noted that the doses of glucagon ily used as the *in vivo* expts referred to above eed those obtained during physiological con-

results show then, that of the 9 peptides
1 only VIP is able to directly influence vaso-
2 cuth muscle tone *in vitro* in a way that seems
3 able with reported *in vivo* effects of the sub-
4 Any vascular effect mediated by the other
5 let studied is probably produced by indirect

6 body was supported by grants from the Swedish
7 al Research Council (04X-00028 and 04X 2210), the

Medical Faculty University of Lund, and AB Hissle
Göteborg. We especially acknowledge the skilled techni-
cal assistance provided by Monica Lundahl, Monica
Hedenholm and Lea Nordström.

REFERENCES

- BERTAQUINI, G. 1976 Aeth polypeptides of non-mammalian origin. *Pharmacol Rev* 28, 127-177.
BLOOM, S. R. & EDWARDS, A. V. 1960 Effects of exogenous stimulation on the release of vasoactive intestinal peptide from the gastrointestinal tract in the calf. *J Physiol (Lond.)*, 99, 437-452.
COCKS, T. & BURNSTOCK, W. 1979 Effect of neuronal polypeptides on intestinal smooth muscle: a comparison with non-adrenergic non-cholinergic nerve stimulation and ATP. *Eur J Pharmacol* 54, 251-259.
EKLUND, S., JODAL, H., LUNDQVIST, O. & SJÖQVIST, A. 1979 Effects of vasoactive intestinal

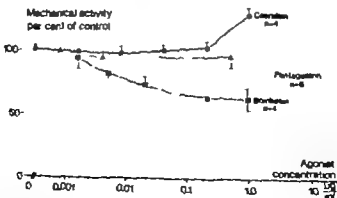


Fig. 7. Mechanical activity of portal veins on exposure to carbachol, pentagastrin and bombesin. Recovery activities after washout were for carbachol $103 \pm 4\%$, for pentagastrin $72 \pm 7\%$ and for bombesin $76 \pm 10\%$.

- polypeptide on blood flow, motility and fluid transport in the gastrointestinal tract of the cat. *Acta Physiol Scand* 105: 461-468.
- EULER U & GADDUM J H 1931 An unidentified depressor substance in certain tissue extracts. *J Physiol (Lond)* 72: 74-87.
- FAHRENKRUG J, HAGLUND U, JODAL M, LUNDGREN O, OLBE L & SCHAFFALITZKY de MUCKADELL O B 1978 Nervous release of vasoactive intestinal polypeptide in the gastrointestinal tract of cats. Possible physiological implications. *J Physiol (Lond)* 284: 291-305.
- FASTH S, FILIPSSON S, HULTÉN L & MAR TINSSON J 1973 The effect of gastrointestinal hormones on small intestinal motility and blood flow. *Experientia (Basel)* 29: 982-984.
- HALLBERG D & PERNOW B 1975 Effect of substance P on various vascular beds in the dog. *Acta Physiol Scand* 97: 277-285.
- HELLSTRAND P 1979 Mechanical and metabolic properties related to contraction in smooth muscle. *Acta Physiol Scand Suppl* 464: 1-54.
- JÄRHULT J, HELLSTRAND P & SUNDLER F 1980 Immunohistochemical localization and vascular effects of vasoactive intestinal polypeptide in skeletal muscle of the cat. *Cell Tiss Res* 207: 55-64.
- KACHELHOFFER J, ELOY M R, POUSSE A, HOHMATTER D & GRENIER J F 1974 Mesenteric vasomotor effects of vasoactive intestinal polypeptide. Study on perfused isolated canine jejunal loops. *Pflug Arch* 352: 37-46.
- KANETO A, KANEKO T, KAJINUMA H & KOSAKA K 1978 Effects of substance P and neurotensin infused intrapancreatically on glucagon and insulin secretion. *Endocrinology* 102: 393-401.
- LJUNG B 1970 Nervous and myogenic mechanisms in the control of a vascular neuroeffector system. *Acta Physiol Scand Suppl* 349: 33-68.
- LÖFSTRÖM B, PERNOW B & WAHREN J 1965 Vasodilating action of substance P in the human forearm. *Acta Physiol Scand* 63: 311-324.
- MACFERRAN S N & MAILMAN D 1977 Effects of glucagon on canine intestinal sodium and water fluxes and regional blood flow. *J Physiol (Lond)* 266: 1-11.
- MOORE A F 1979 The interaction of enkephalins with substance P on vascular smooth muscle. *Res Comm Chem Pathol Pharmacol* 23: 233-4.
- NILSSON G, DAHLBERG K, BRODIN E, SUNDLER F & STRANDBERG K 1977 Distribution and constrictor effect of substance P in guinea pig tracheobronchial tissue. In *Substance P* (ed U S Euler & B Pernow), pp 75-81. Raven Press, New York.
- PERNOW B 1960 Effect of substance P on muscle. In *Polypeptides which affect smooth and blood vessels* (ed M Schechter), p 18. Monograph, Elmsford, New York.
- PERNOW B & ROSELL S 1975 Effect of substance P on blood flow in canine adipose tissue and muscle. *Acta Physiol Scand* 93: 139-141.
- RICHARDSON P D I & WITHERINGTON P G 1974 Glucagon inhibition of hepatic arterial response to hepatic nerve stimulation. *Am J Physiol* 226: H647-H654.
- ROSS G 1970 Regional circulatory effects of glucagon. *Br J Pharmacol* 38: 733-74.
- SAID S & MUTT V 1970. Potent peptide splanchnic vasodilator peptide from serum. *Nature* 225: 863-864.
- SAMNEGÅRD H, THULIN L, ANDREY TYDÉN G, HALLBERG D & EFENDIĆ S 1973 Circulatory effects of somatostatin in man and dogs. *Acta Chir Scand* 145: 709-1.
- SCHAFFALITZKY de MUCKADELL O B I, FAHRENKRUG J & HOLST J J 1977 Effect of vasoactive intestinal polypeptide (VIP) on the stimulation of the vagal nerves. *Gastroenterology* 72: 373-375.
- SHOEMAKER W C, ITALLIE, van T J & WALKER W F 1949 Measurement of hepatic glucose output and hepatic blood flow in response to glucagon. *Am J Physiol* 156: 315-318.
- THULIN L 1973 Effects of gastro-intestinal peptides on hepatic bile flow and splanchnic circulation. *Acta Chir Scand Suppl* 441: 1-31.
- TIBBLIN S, ROCK N G & SCHENK W G 1970 Splanchnic hemodynamic responses to pyloric constriction. *Arch Surg* 100: 84-89.
- TYDÉN G, SAMNEGÅRD H, THULIN L, MUHRBECK O & EFENDIĆ S 1979 Circulatory effect of somatostatin in anaesthetized man. *Acta Physiol Scand* 111: 443-446.
- VAGNE M, STENING G F, BROOKS F, GROSSMAN M I 1968. Synthetic secretin: comparison with natural secretin for potency and effects on physiological actions. *Gastroenterology* 54: 767.
- WAHREN J, EFENDIĆ S, LUFT R, HÄFELDT L, BJÖRKMAN O & FELIG P 1977 Influence of somatostatin on splanchnic metabolism in postabsorptive and 60-hour fasted man. *J Clin Invest* 59: 299-307.

Intestinal tissue osmolality, water and electrolyte transport in the cat small intestine at varying luminal osmolalities

AXEL HALLBACK, MATS JODAL and OVE LUNDGREN

Department of Physiology, University of Göteborg, Sweden

HALLBACK, A., JODAL, M. & LUNDGREN, O. Vileous tissue osmolality, water and electrolyte transport in the cat small intestine at varying luminal osmolalities. *Acta Physiol Scand* 1980, 110, 95-100. Received 27 Dec. 1979. ISSN 0001-6772. Department of Physiology, University of Göteborg, Sweden.

Vileous tissue osmolality and net transport for water, sodium, potassium and chloride were determined in the feline small intestine when exposing the mucosa to solutions with different osmolal concentrations (0, 100, 315 and 600 mmol/l). Tissue osmolality at the vileous tip varied with luminal osmolality. At the vileous base, on the other hand, tissue osmolality remained around the plasma osmolality regardless of the osmolality of the luminal fluid. Transport rates for water were affected in the way predicted from the lumen to tissue osmolality difference. A net flux from tissue to lumen was always recorded for the studied electrolytes. The hydraulic conductivity (L_p) of the intestinal epithelium with dilated intercellular spaces was estimated from the present results to be around $30 \cdot 10^{-12}$ cm cm^{-2} Pa^{-1} s^{-1} . When the intercellular spaces were collapsed L_p was estimated to be $11 \cdot 10^{-12}$ cm cm^{-2} Pa^{-1} s^{-1} .

Key words: Intestinal water transport, intestinal electrolyte transport, hydraulic conductivity of intestinal epithelium.

Transfer of water from the intestinal lumen into the tissue is in vivo accomplished by the action of a hyperosmolar compartment in the core, secondary to the action of the interstitial osmotic multiplier (Haljamae et al. 1973; et al. 1978). The tissue osmolality at the tip of villi has been estimated to about 1 000 mOsm/l H_2O in cat (Jodal et al. 1978) and to about 800 mOsm/l H_2O in man (Hallback et al. 1978) when exposing the intestinal mucosa to an isotonic solution containing sodium and glucose. In a previous study (Hallback et al. 1979) it was shown that varying the sodium and/or glucose concentration in the luminal solution altered the tissue osmolality in a manner consistent with known cellular transport mechanisms in the enterocytes. The lumen to tissue osmolality influenced in turn the water uptake from the gut lumen. In the present study the osmolality in the intestinal lumen was varied by exposing the mucosa to different mannitol solutions, in order to analyze the effect of such changes on vileous tissue osmolality and net transport of water and electrolytes.

METHODS

A. Operative procedures

The experiments were performed on cats anesthetized with chloralose (50 mg/kg b.w.) after ether induction. The animals had been deprived of food for 4 h and had no obvious signs of intestinal infection. The operative procedure was largely similar to those described by Jodal et al. (1975) which paper should be consulted for details. Mean arterial pressure was measured from the left femoral artery by pressure transducer (Statham P23AC). The splanchnic nerves were divided bilaterally and the cholinergic influence was eliminated by atropine administration, 1 mg/kg b.w. Acidosis was prevented by slow continuous infusion (0.1 ml/min) of bicarbonate-glucose solution in the left femoral vein (Haglund & Lundgren 1972).

B. Recording of intestinal net water transport and experimental procedures

Net water absorption or secretion was determined according to the method described by Jodal et al. (1975). Four segments were used in randomized manner. A recirculating system was coupled to the lumen of each intestinal segment. The volume change of this system was continuously recorded with volume transducer connected to the system via Y-tube. Provided no motility occurred the recorded volume changes reflected net water absorption.

Table 1 Net rates of water and electrolyte transport in the jejunum of cats when exposed to segments to four solutions with different concentrations of mannitol

(-) denotes a transport from tissue to lumen. Mean \pm S.E.

Mannitol conc (mmol/l)	Water $\mu\text{l}/\text{min} \times 100 \text{ cm}$	Sodium $\mu\text{mol}/\text{min} \times 100 \text{ cm}^2$	Potassium	Chloride
0 (n=6)	-58 ± 6	-34.1 ± 5.8	-1.46 ± 0.41	-41 ± 6
100 (n=7)	173 ± 76	-20.4 ± 5.9	-1.37 ± 0.19	-17 ± 8
315 (n=7)	60 ± 36	-19.0 ± 7.3	-0.79 ± 0.23	-29 ± 8
600 (n=5)	-158 ± 5	-28.7 ± 11.5	-0.69 ± 0.19	-39 ± 1

Statistically significant from 315 mmol/l

or secretion. The intraluminal pressure at the outflow end of the segment was kept at 1 cm H₂O and the intestinal segment was perfused at a constant rate of 1 ml/min by means of a roller pump (Model mp-4 Ismatec SA Zurich Switzerland). Recirculation was prevented by including a large reservoir (700-1000 ml) in the circulating system. The temperature of the perfusate entering the segment was continuously monitored with a thermocoupled thermometer (Electrolab Copenhagen) and kept at 38°C by a heating pad. The temperature close to the intestinal segments was kept constant by a lamp connected to a temperature controlled relay.

After a perfusion period of 30 min samples of the perfusate entering and leaving each segment were collected. Immediately thereafter the segments were rapidly excised and momentarily frozen in isopentane precooled to about -150°C in liquid nitrogen.

C. Biochemical and osmotic measurements

In order to calculate the net transport of electrolytes across the intestinal epithelium, samples (about 5 ml) were taken from the perfusates entering and leaving the intestinal segments. The samples were collected in plastic tubes and the sodium and potassium concentrations were determined with a flame photometer (Eppendorf) while the chloride concentration was determined with a chloride meter (Corning Eel 970 Chloride Meter Halstead Essex England). The osmolalities of the samples were determined with an osmometer (Advanced Instruments Inc model 68-311AS).

Intestinal net transport of sodium, potassium and chloride was calculated as described by Hallbeck et al (1979b). Net transport rates were related to serosal area and expressed as $\mu\text{l}/\text{min} \times 100 \text{ cm}^2$ serosal surface (water) or as $\mu\text{mol}/\text{min} \times 100 \text{ cm}^2$ serosal surface (electrolyte).

D. Cryoscopic technique

1. General procedure. The cryoscopic method with calibration procedure was presented in detail by Jodal et al (1978). Briefly, the frozen intestinal segment was cut obliquely, a cryostat kept at -5°C so as to give 15 μm thick cross-sections at different levels along the villus. The tissue sections were embedded in kerosene between glass plates and transferred to a temperature-controlled unit consisting of an aluminium block mounted on a Zeiss

standard microscope connected with an automatic (Rohot Star 50). The temperature of the alcohol could be set at any temperature between -4 and +0.5°C. Thawing of the intestinal tissue was done stepwise increases of tissue temperature, back up automatically with a 5 min equilibration period at each step. At the end of each period the tissue was photographed.

2. Determination of tissue osmolality. The temperature at which the last ice crystals thawed in a given section was taken as its melting point. The lower point determined in this study was considered a measure of the villous core osmolality. The magnification (about 50 times) was too low to allow any detection of intracellular ice crystals in the villous tissue. Percentage of melted villous tissue for each level was determined from the photographs. The data were fed into a computer which calculated the osmolality at 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% as well as the mean osmolality between 5 and 40 and 5 and 100% villous length as described in detail by Jodal et al (1978).

E. Histological procedure

In three experiments a rectangular piece (1 x 3 cm) of perfused intestinal segment was mounted on a fixed 10% neutral formaline. The block was used for routine paraffine embedding, cut at about 6 μm and stained according to von Gieson.

F. Solution

The intestinal mucosa was exposed to mannitol of four different concentrations, i.e. 0, 100, 315 and 600 mmol/l. In all experiments only one type of solvent perfused through each segment.

G. Statistics

Statistical significance was tested using the sign Wilcoxon nonparametric test (Siegel 1956).

RESULTS

Table 1 gives the results on net water and net electrolyte transport rate in the different types of

— T_p and mean osmolality in various portions of intestinal villi expressed in mOsm/kg H_2O Mean

	Tip	From 5 to 30% of villous length	From 5 to 50% of villous length	From 5 to 100% of villous length
(6)	23. \pm 9	254 \pm 8	268 \pm 7	282 \pm 6
(7)	253 \pm 9	271 \pm 15	272 \pm 8	283 \pm 4
(10)	368 \pm 9	346 \pm 7	336 \pm 6	327 \pm 4
(17)	507 \pm 1	430 \pm 11	403 \pm 10	367 \pm 6

results. The expected decrease in net water J_w with increasing mannitol concentration in luminal perfusate is evident, although the water transport rate from solution devoid of mannitol is lower than that from a 100 mmol/l mannitol one. The electrolytes studied all exhibited a net flow from tissue to lumen. Sodium and chloride secret rates showed no consistent change when raising the luminal perfusate osmolality while the rate of potassium from the tissue decreased with raising lumen osmolality. The villous core osmolality as estimated with the wick method is shown in Table 1 (villous tip osmolality and mean osmolality at varying levels of the villi) and in Fig. 1 (osmolality gradient in villi). The exposure of the intestinal mucosa to hypo-osmotic solutions produced a villous tip hypo-osmolarity particularly in the villous tips,

while osmolality approached isotonicity in the basal villous portions. On the other hand with the hyper-tonic mannitol solution in the intestinal lumen tissue osmolality increased in the upper villous portions.

Knowing tissue and luminal osmolality it was possible to estimate the transepithelial osmotic gradient for each experiment in Fig. 1 the lumen to tissue osmolality difference is plotted against net water transport rate for all groups except the one with no mannitol. When calculating lumen osmolality a mean value of the entering and leaving fluid osmolality was estimated assuming an exponential change in the intestinal segment (Soergel et al 1968). In this figure a corresponding calculation is also included using the values reported by Hallböök et al (1979b). In that study the tissue osmolality was varied by perfusing the intestinal lumen with isotonic solutions of different sodium and glucose concentrations.

The values for tissue osmolality were those calculated between 5 and 30% of villous length (Table 1) as water absorption was assumed to occur mainly across this part of the villus. The results of the two studies form two parallel biphasic curves that of the present study being placed to the left of the one of Hallböök et al (1979b). The two phases of the curve based on the results of Hallböök et al were constructed using the methods of least squares. These lines are indicated in the Figure.

The slope of the curves represent the amount of fluid transported per unit osmolality difference, i.e. they reflect the hydraulic conductivity (L_p) of the intestinal epithelium. In table 3 the corresponding hydraulic conductivities are calculated assuming a reflection coefficient of 1 for mannitol and sodium chloride the latter probably being the main solute in intestinal interstitial fluid. Water absorption is assumed to take place across the upper third or half of the villus. The corresponding values of the ex-

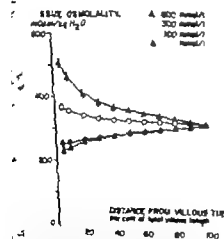


Fig. 1. Villous tissue osmolality when varying the mannitol concentration of the solution perfusing the lumen decreases \pm S.E. At many points the S.E. is so small that it falls below the area of the symbol for number of experiments, see Table 2.

Table 3 Hydraulic conductivity (L_p) of the intestinal epithellum in $\text{cm} \times \text{s}^{-1} \times \text{Pa}^{-1}$ as calculated from slopes of the curve illustrated in Fig. 2

The L_p values have been estimated assuming water absorption to occur across the upper third or half of the villi

	Upper 30% of villus length		Upper 50% of villus length	
	Steep slope	Flat slope	Steep slope	Flat slope
Present study	31×10	14×10	19×10	8×10^{-10}
Hallbäck et al (1979b)	6×10	18×10	22×10	16×10

periments reported by Hallbäck et al (1979b) are also included in Table 3

The histological investigations revealed damaged epithelial cells restricted to the very tips of most villi in intestinal segments exposed to solutions containing no mannitol and in some villi in the 100 mmol segments. No histological damage was observed in segments perfused with 315 or 600 mmol mannitol solutions

DISCUSSION

The present study demonstrates that varying the osmolality of the intestinal lumen within wide limits in some way affects markedly the tissue osmolality in upper parts of the villus. At the base of the villus, on the other hand, tissue osmolality approached that of arterial plasma regardless of which fluid that was placed in the intestinal lumen. Hence the plasma osmolality in the vessels draining the intes-

tinal villi is always close to isotonicity. This is considered as one functional effect of the countercurrent exchanger (Lundgren 1967): the subepithelial capillaries/veins in the villi equilibrate with the isotonic blood in the distally situated supplying arterial vessel. Had the capillaries/veins been located at some distance from supplying arterial vessels, the venous plasma osmolality had much more closely reflected that of intestinal contents. Hence the intestinal countercurrent exchanger acts as a damping device with respect to the luminal osmolality, a function must be of particular importance in the duodenum.

The presence of osmoreceptors in the intestinal mucosa which influence the rate of gastric emptying has been inferred from a large variety of experiments (cf. Hunt & Knox 1968). The demonstration in this and previous studies (Jodal et al 1979; Hallbäck et al 1979) that the intestinal concentration of sodium and/or glucose or the intestinal lumen

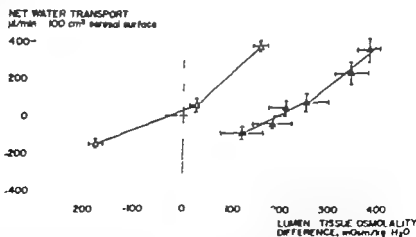


Fig. 2 The relationship between net water transport and lumen to tissue osmolality difference. The right curve was constructed from the results reported by Hallbäck et al (1979b) using the method of least squares. The steep part of this curve was calculated from the three highest osmolality difference values and the flat part from the four lowest values. Bars denote S.E. For number of observation see Table 1 and Hallbäck et al (1979b).

particularly influence villous osmolality suggest that the osmoreceptors for gastric juice are located in the core of the villus. This has, however, never been tested since lumen osmolality has not been determined in the regions of gastric emptying.

It illustrates that net water transport across intestinal epithelium is not linearly related to the osmotic lumen to tissue osmolality difference phenomenon has been demonstrated for several epithelia and has been named 'rectification of water flow'. As discussed by House (1974) a likely explanation is the changes in morphology of the intestinal epithelium induced by the direction of net water flow. When the tissue is hypertonic in relation to lumen and consequently π into the tissue, the lateral intercellular spaces are distended (Tomasson & Dobson 1970; Dobson et al. 1974). When water moves in opposite direction the intercellular spaces collapsing increased flow resistance (right part of Fig. 3 in McElligott et al. 1975). Based on this it is proposed that the hydraulic conductance during absorption is represented by the decrease of the upper steep curves in Fig. 1. Summing the L_p value of the intestinal epithelium with the intercellular spaces is reflected in the decrease of the two lower curves of Fig. 1 (see also Fig. 2). It is further proposed that the L_p value for net water absorption, i.e. about 30×10^{-12} cm³ s⁻¹ Pa⁻¹ reflects almost exclusively L_p of the junctions. This proposal is based on two assumptions: (1) in this situation water enters the villi exclusively through the tight junctions and (2) distended parts of intercellular spaces do not offer substantial flow resistance compared with tight junctions.

Overall hydraulic conductivity of the intestinal epithelium has been measured with a method similar to one used in this study by Fordtran et al. (1965) and Soergel et al. (1968) although these authors considered the villous tissue osmolality to be low. Recalculating their results to the units for use in this study and using the anatomical ratios for the human intestine reported by Jørgensen et al. (1968) one arrives at the values of 34×10^{-12} cm³ s⁻¹ Pa⁻¹. In the two above-mentioned investigations. These values were based on experiments where a mannitol induced net secretion of fluid was present (right part of Fig. 3) and could consequently be compared to the value

around 15×10^{-12} cm³ s⁻¹ Pa⁻¹ found in the present study.

The curves relating lumen-tissue osmolality difference to net water transport (Fig. 2) do not coincide the curve of the present experiments being located to the left of that constructed from the results of Hallböök et al. (1979b). It is not possible from the present results to explain this difference between the two curves, but at least two contributing factors exist. First, the reflection coefficient of mannitol (in the lumen) is probably higher than that of sodium chloride (cf. Fordtran et al. 1965) which constitutes most of the hyperosmolality in the intestinal villi. Second, in the experiments of Hallböök et al. an increased water flux from tissue to lumen in e.g. the crypts may explain why their curve is placed more to the right.

The present research was supported by grants from the Swedish Medical Research Council (14X 2835) from the Swedish Society for Medical Sciences, from Magnus Bergvall Stiftelse and from the Faculty of Medicine, University of Göteborg.

REFERENCES

- DIBONA, D. R., CHEN, L. C. & SHARP, G. W. G. 1974. A study of intercellular spaces in the rabbit jejunum during acute volume expansion and after treatment with cholera toxin. *J. Clin. Invest.* 53, 1300-1307.
- FORDTRAN, J. S., RECTOR, F. C., J. EWTON, M. F., SOTER, N. & KINNEY, J. 1965. Permeability characteristics of the human small intestine. *J. Clin. Invest.* 44, 1935-1944.
- HALLBÖÖK, U. & LUNDGREN, O. 1972. Reactions within consecutive vascular sections of the small intestine of the cat during prolonged hyponatremia. *Acta Physiol. Scand.* 84, 151-163.
- HALLBÖÖK, U., JODAL, M. & LUNDGREN, O. 1973. Countertransport mechanism of sodium in intestinal villi during absorption of sodium chloride. *Acta Physiol. Scand.* 89, 580-593.
- HALLBÖÖK, U., JODAL, M. & LUNDGREN, O. 1979. Importance of sodium and glucose for the establishment of villous tissue hyperosmolality by the intestinal countertransport multiplier. *Acta Physiol. Scand.* 107, 89-96.
- HALLBÖÖK, U., JODAL, M., SJÖQVIST, A. & LUNDGREN, O. 1979b. Villous tissue osmolality and intestinal transport of water and electrolytes. *Acta Physiol. Scand.* 107, 1-126.
- HALLBÖÖK, U., HULTEN, L., JODAL, M., LINDHAGEN, J. & LUNDGREN, O. 1978. Evidence for the existence of countertransport exchangers in the small intestine in man. *Gastroenterology* 74, 683-690.
- HOUSE, C. R. 1974. Water transport in cell and tissues. E. Arnold Ltd. London.

- HUNT J N & KNOX M T 1968 Regulation of gastric emptying. In *Handbook of physiology* Section 6 (Alimentary canal) vol IV pp 1903-1916
- JODAL, M. HALLBÄCK D A & LUNDGREN O 1978 Tissue osmolality in intestinal villi during luminal perfusion with isotonic electrolyte solutions. *Acta Physiol Scand* 10, 94-107
- JODAL, M. HALLBÄCK D A SVANVIK J & LUNDGREN O 1975 A method for the continuous study of net water transport in the feline small bowel. *Acta Physiol Scand* 95 441-447
- LUNDGREN O 1967 Studies on blood flow distribution and countercurrent exchange in the small intestine. *Acta Physiol Scand Suppl* 303
- McELIGOTT T F BECK I T DANDY THOMPSON III 1975 Correlation of changes at different levels of the jejunum of positive and negative net water transport *in vitro*. *Can J Physiol Pharmacol* 53 439-449
- SOERGEL, K H WHALEN G E & HALL 1968 Passive movement of water and ions in the human small intestinal mucosa. *J Appl Physiol* 4 40-48
- TOMASINI J T & DOBBINS W O 1975 Mucosal morphology during water and electrolyte absorption. A light and electron microscopic study. *Dis* 15 226-238

Concentration of immunoreactive calcitonin of prostatic origin in human semen

SJOBERG S ARVER and E. BUCHT

Department of Endocrinology, Karolinska Hospital and Reproductive Physiology Unit
Department of Physiology, Karolinska Institute, Stockholm, Sweden

It was demonstrated that only about 4% of calcium in human semen is present in ionized form (Arver & Sjöberg 1980). The fact that seminal calcium concentration is about 3 times that of blood suggests the existence of a mechanism for regulation of Ca in seminal fluid. Calcitonin might play a role in this connection. For this and also since CT is known to increase the excretion of Ca (Hjern, Granberg & Sjöberg 1979) we measured the apparent CT concentration in human semen to see whether it might be positively related to Ca concentration.

Semen samples were obtained from 18 men at the reproductive physiology unit for a barium test. In addition, sperm free seminal fluid was obtained from 4 vasectomized men and split ejaculates from 6 volunteers (medical students). 13 of the 18 samples with normal recording concerning morphology and seminal plasma composition (according to Eliasson 1975) were regarded as a reference group which was compared to 5 samples with apparently subnormal prostatic secretion (Zinc <1.0 mM) and to the samples from the vasectomized men having normal composition of the seminal plasma. In another series of 10 men's split ejaculates (Kvist 1980) were

employed to obtain semen fractions with either predominantly prostatic (portion 1) or vesicular gland (portion 2) contributions to the seminal plasma.

CT was measured by radioimmunoassay using rabbit antibodies to synthetic human calcitonin (Immunonuclear Corp. USA). Normal human serum CT measured with this method had given a mean value of 4 pg/ml (range <1–100). Ca, Mg and Zn were determined with atomic absorption spectrophotometry, acid phosphatase activity and fructose concentration with colorimetric methods (cf Eliasson 1975). For descriptive statistics mean and S.D. are given. Correlation ratings were estimated as Pearson's product moment correlation. Student's *t*-test was used for group and paired analyses (cf Colton 1974).

In the reference group mean seminal plasma CT was 1771 pg/ml (range 1000–2880, S.D. ± 612) i.e. about 40 times serum CT. The values for other semen variables are presented in Table 1. CT was positively correlated to Ca, Mg, Zn and acid phosphatase ("prostatic markers") but not to fructose ("vesicular gland marker") (Table 2). Furthermore subdivision of the reference samples into a low (<16 mM) and high (>16 mM) fructose group did not reveal any CT difference between the two

Table 1. Concentrations of calcitonin, total alkaline phosphatase, zinc and acid phosphatase activity and fructose in seminal plasma from 13 subjects

	Mean \pm S.D.	Range
Calcium, pg/ml	1771 \pm 612	1000–2880
Magnesium, nmol/l	6.1 \pm 1.6	3.4–8.7
Zinc, nmol/l	4.4 \pm 1.27	2.0–6.9
Acid phosphatase, U/l	5 \pm 1.0	1.3–4.6
Fructose, mmol/l	14.9 \pm 7.1	7.7–33.9
Total, mmol/l	15.7 \pm 6.3	7.3–30.8

Table 2. Correlation coefficients between calcitonin and secretory products of prostatic and vesicular origin

Correlation to	<i>P</i>	Origin
Calcium	0.63	<0.001
Magnesium	0.77	<0.001
Zinc	0.80	<0.001
Acid phosphatase	0.75	<0.001
Fructose	0.16	<0.2

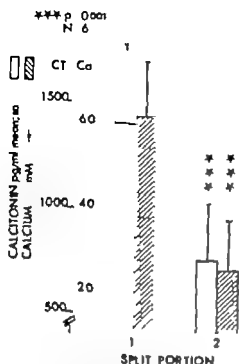


Fig. 1 Calcitonin and calcium concentration in portions 1 ("prostatic fluid") and portions 2 ("vesicular fluid") of split ejaculates of human semen.

The group of semen samples with subnormal concentrations of Zn and other prostatic markers contained significantly less CT than the reference group ($P < 0.01$, $df = 16$). In contrast seminal fluid from the vasectomized men contained as much CT as the reference group and also had normal concentrations of Ca, Zn and other prostatic markers.

The split ejaculate experiments revealed obvious differences between portions 1 and 2. In portion 1 ("prostatic fluid") the concentrations of CT, Ca (Fig. 1), Mg, Zn and acid phosphatase activity were conspicuously higher ($P < 0.001$, $N = 6$) than in portion 2 ("vesicular gland fluid").

DISCUSSION

The present demonstration of high CT immunoreactivity in human semen may provide an explanation of the steep gradients between blood serum and semen Ca and between ionized Ca and total Ca in the seminal plasma (Arver & Sjöberg 1980). The presence of large amounts of CT somewhere in the male accessory glands may be the ultimate cause of the conspicuous Ca transfer to the seminal fluid and may also explain why CT per se is also transported in large amounts in this fluid. Here the hormone may counteract the ionization of

Ca and influence the exchange of Ca between spermatozoa and their external environment. The possibility that seminal plasma CT might come from a higher level of the male reproductive system (testis/epididymis) seems to have been ruled out by the observation that also ejaculates from vasectomized men contained as much CT as in semen samples.

CT has been found to stimulate not only renal but also renal Mg excretion (cf. Munson 1976). In the present study seminal CT was correlated to Mg and Zn. Therefore CT may facilitate only the transfer of Ca but also of other cations to the seminal fluid.

Regarding the source of seminal CT the experiments strongly suggest that the hormone is added to the semen by prostatic secretion. The determinations revealed a positive correlation between the concentrations of CT and prostatic markers in semen. Furthermore in the split ejaculate experiments the concentration of CT was considerably higher in the fractions of the ejaculate obtained by prostatic fluid than in those of predominantly vesicular gland fluid. How remains to be elucidated whether CT might be produced by some cells in the prostate gland. The high amounts of CT in its fluid is a manifest prostatic trapping of circulating CT.

We are greatly indebted to Drs R. Thoren and L. Löf for generously supplying us with laboratory equipment. Financial support was obtained from Lundberg's Foundation.

REFERENCES

- ARVER B & SJÖBERG H E 1980. Ionized calcium in human seminal plasma. *Hormone Metab Res* 12: 101-104.
- COLTON T 1974. *Statistics in medicine*. Little, Brown, Boston.
- ELIASSON R 1975. Analysis of semen for infertility. In: *Infertility* (ed. S. J. Behrman and R. W. Judd), pp. 691-713. Little Brown & Co., Boston.
- HJERN H, GRANBERG P-O & SJÖBERG H E 1972. Acute effects of porcine calcitonin on electrolyte excretion in man. *Proc Vth Internat Nephrol* 1: 109-113.
- KVIST U 1980. Reversible inhibition of chromatin decondensation (NCD) ability of permeant ions induced by prostatic fluid. *Acta Scand* 109: 73-78.
- MUNSON P L 1976. Physiology and pharmacology of thyrocalcitonin. In: *Handbook of physiology* 7: Endocrinology (ed. F. H. Acheson & G. Greeb), pp. 441-464. Amer Physiol Soc, Wash DC.

Is the central pattern generation for locomotion in lampreys depend on glycine inhibition?

J. GRILLNER and PETER WALLÉN

Department of Physiology III, Lidönggatan 1, Karolinska Institute, S-11433 Stockholm, Sweden

Knowledge about the general organizational principles underlying the neural control of locomotion in vertebrates has expanded markedly during a few years (cf. Stent 1978; Stok & Orlovsky 1975). On the other hand, the exact neural circuitry controlling locomotion or any similar rhythmic behaviour is still unknown. In vertebrate networks are required to elucidate how such circuits may operate. Such models can later be tested on higher vertebrates to find if they apply or how they may have become modified. We have used one simple vertebrate preparation, the lamprey spinal cord, in which the rhythmic activity normally generating swimming can be elicited also under in vitro conditions (Poon & Rovainen 1977; Cohen & Wallén 1978; Wallén 1978).

Several modes of operation have been tested for such central pattern generators (CPGs) (Gurfinkel & Stok 1973; Kling & Székely 1976; Selverston 1976; Getting et al. 1980). Some are based on burst-generating circuits of excitatory neurons, others exclusively on inhibitory neurons, and still others on a mixture of the two. In order to discriminate between the first and the last two possibilities the present experiments were designed. As a result we come to seek evidence for the possible inhibitory transmitters involved. Homma & Rovainen (1978) have shown that the putative inhibitory transmitters glycine and γ -amino-butyric acid (GABA) (cf. Krnjević 1974 and Martin et al. 1970) both act in the lamprey spinal cord. Strychnine (a glycine receptor antagonist) and bicuculline (a GABA receptor antagonist) block their action in a specific manner (cf. Krnjević 1974; Homma & Rovainen 1978; Curtis et al. 1968). Both amino acids cause a marked increase of membrane conductance in Cl^- -ions, associated with a neuronal hyperpolarization (Homma & Rovainen 1978).

The lamprey spinal cord is flat (200 μ m thick, 2 mm long) and does not contain blood vessels *in vivo* as it is perfused from the cerebrospinal fluid, which makes it suitable for *in vitro* experiments. The spinal cords of adult lampreys are dissected in pieces of 10-25

segments (notochord still attached) and placed in an *in vitro* chamber and pinned down in "sylvard". The cord was superfused with lamprey physiological solution (Wickelgren 1977) and the activity of the ventral root was recorded with suction electrodes. After application of D-Glutamate (0.3-1.2 mM) in the bath, "active swimming starts" (Poon 1980; Cohen & Wallén 1978, 1979), i.e. bursting motor output in each segment (Fig. 1A) with adequate intersegmental coordination. The temperature was kept at 7-8°C. The rate of bursting may be markedly dependent on the pool temperature (Fig. 1F and Legend).

The frequency of the rhythmic alternating activity (Fig. 1A) increases if strychnine (0.5-3 μ M) is applied in the bath (Fig. 1B and E) to become irregular and change over to tonic efferent activity with a higher dose (Fig. 1C). As the rate of activity is modified, strychnine must interact with the pattern generator itself or the spinal neurons controlling it. The amount of strychnine used is low (Fig. 1B). It is in the lower range of doses used by Homma & Rovainen (1978) to demonstrate competitive antagonism between glycine and strychnine in this preparation. This finding thus suggests that neurons releasing the putative transmitter glycine are active in the pattern generation. If so, it would be expected that glycine itself should also influence the bursting. Glycine may cause both marked irregularities in the burst pattern and a decreased rate (50 μ M in Fig. 1D). At somewhat higher dosages all activity ceases. Homma & Rovainen (1978) used concentrations of up to 1 000 μ M glycine to obtain maximal conductance effects. When applying glycine in the bath it will of course act on all neurons with glycine receptors. The effect of strychnine on the other hand will only be manifested in those synapses which are active and release glycine in this particular experimental condition (active swimming).

The GABA antagonist bicuculline should in the same way as strychnine affect the activity of the pattern generator if presumed GABA neurons were important in this context. Bicuculline (Fig. 1A, B, E) has no effect in a dosage which will block the

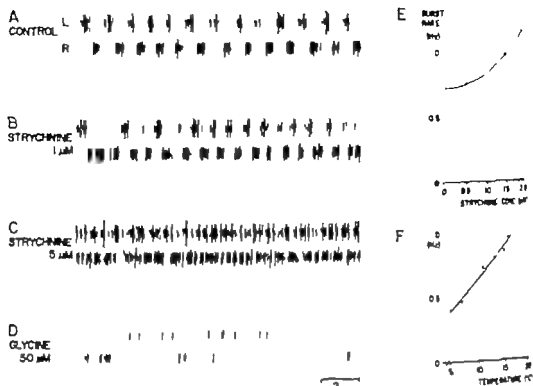


Fig. 1. The effect of glycine and strychnine on the burst pattern during fictive locomotion. A shows the burst recorded in one ventral root on the left (L) and one on the right (R) side when using normal physiological (2 mM) glutamate added (control for B–D). In B and C 1 and 5 μ M strychnine was added respectively. D shows the inhibitory effect of 50 μ M glycine. Note time calibration. The graph in E shows the burst frequency at different concentrations of strychnine for a different preparation and F the effect of different bath temperatures on the burst. In this graph the temperature was progressively increased from 5 to 20°C within 11 min. The sensitivity to seems to vary i.a. between preparations.

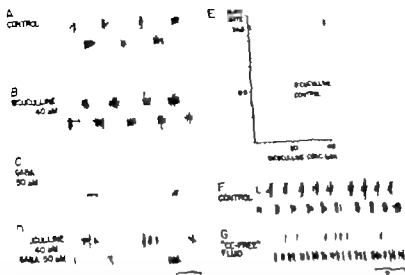
conductance changes evoked by GABA (Homma & Rovainen 1978; see also Fig. 2 D). Already at 1.4 μ M bicuculline their GABA effects were antagonized. It thus appears that synapses with receptors blocked by bicuculline are not of critical importance in producing this burst pattern. Application of GABA to the bath can reveal if pattern generator neurons (including those controlling the CPG) have GABA receptors. There is a marked reduction of the burst rate already with 50 μ M GABA (Fig. 2 C). This effect of GABA is antagonized by bicuculline (Fig. 2 D). Neurons releasing GABA are thus apparently not of importance for the generation of the present fictive locomotion, but if such neurons were to become active in some other behavioural condition they could influence the pattern generation.

To further test the possibility that inhibitory mechanisms are involved in the pattern generation we have blocked the inhibition associated with a conductance increase to Cl^- ions (e.g. GABA and glycine; Homma & Rovainen 1978). This can be accomplished by utilizing a physiological fluid in

which the chloride content has been reduced by substituting Cl^- with SO_4^{2-} (Calabrese 1978; Homma & Rovainen 1978). The application of a solution gives practically no effect on the action potentials or on the membrane potential (Homma & Rovainen 1978). Glycine does not give rise to a transient depolarization but there is no conductance increase at all (presumably intracellular Cl^- concentration approaches zero). Application of such Cl^- free physiological solution (with D-Glutamate) results in a complete down of the burst pattern and a tonic activation (Fig. 3).

In conclusion, neurons releasing a transmitter blocked by strychnine (presumably glycine) are not an active part in producing the burst pattern. In contrast, possible GABA neurons seem to be involved under the present experimental conditions.

This study has been supported by the Swedish Research Council (project no. 3026), Magnus F. Stiftelse and Karolinska Institutet, Sweden.



The effect of GABA, bicuculline and chloride free physiological solution on the burst pattern during fictive locomotion. A shows the control burst pattern as in Fig. 1 (0.5 mM glutamate) and B, C and D the effect of bicuculline (40 µM), GABA (50 µM) and the combined effect of bicuculline and GABA. The effect of GABA is clearly antagonized by bicuculline. The graph in E shows the absence of effect of bicuculline on the burst rate at both low and very high doses. F and G were recorded just prior to the respective bicuculline applications. F is the control burst pattern (0.3 mM Ca^{2+}) (for G which shows the effect of applying Ca^{2+} -free solution. Time calibrations for A-D and F-G respect

REFERENCES

- BRESE, R. L. 1979 The roles of endogenous substrate properties and synaptic interaction in creating the locomotor rhythm of the leech, *Hirudo lewisi*. *J. Exp. Biol.* 82: 163-176.
- HALL, A. H. & WALLÉN, P. 1978 Rhythmic motor activity induced in *in vitro* preparations of lamprey spinal cord. *Neurosci. Lett. Suppl.* 1: S92.
- HALL, A. H. & WALLÉN, P. 1979 A simple vertebrate locomotor model. Fictive swimming in the *in vitro* lamprey spinal cord. *Soc. Neurosci. Abstr.* Vol. 5: 893.
- IS, D. R., HÜSLER, L. & JOHNSTON, O. A. R. 1981 A pharmacological study of the depression of and currents by glycine and related amino acids. *P. Brain Res.* 6: 1-18.
- ITAKI, P. A., LENNARD, P. R. & HUME, R. I. 1980 Central pattern generator producing swimming. *Traverse 1. Identification and synaptic interactions.* *Neurophysiol.* In press.
- LENNARD, P. 1975 Locomotion in vertebrates: Central mechanisms and reflex interaction. *Physiol. Rev.* 55: 27-304.
- PINKEL, V. S. & SHIK, M. L. 1973 The control of posture and locomotion. In: *Motor control* (ed. A. A. Prokhorov, N. T. Tashkov and D. S. Kozlov), pp. 217-244.
- ROVAINEN, C. & ROVAINEN, C. M. 1978 Conductance currents produced by glycine and γ -aminobutyric acid in lamprey interneurons. *J. Physiol.* 279: 31-332.
- ROVAINEN, C. & SZÉKELY, G. 1968 Stimulation of rhythmic nervous activities. I. Function of network with cyclic inhibition. *Kybernetik* 4: 89-103.
- KRNJEVIĆ, K. 1974 Chemical nature of synaptic transmission in vertebrates. *Physiol. Rev.* 54: 418-540.
- MARTIN, A. R., WICKELGREN, W. O. & BERÁNEK, R. 1970 Effects of isotopically applied drugs on spinal interneurons of the lamprey. *J. Physiol.* 207: 657-665.
- POON, M. L. T. 1980 Induction of swimming in lamprey by L-DOPA and amino acids. *J. Comp. Physiol.* 136: 377-344.
- ROVAINEN, C. M. 1977 Proprioceptive interneurons in the lamprey. In: *Neurophysiological mechanisms of locomotion. Satellite Symposium*. 27th Internat. Congr. of Physiol. Sciences, Paris.
- SILVERSTEIN, A. I. 1976 Neural mechanisms for rhythmic motor pattern generation in simple systems. In: *Neural control of locomotion* (ed. R. Herman, S. Grillner, P. Stein and D. Stuart), vol. 18 pp. 377-399. Plenum Press, New York.
- SHIK, M. L. & ORLOVSKY, O. N. 1976 Neurophysiology of locomotor automatization. *Physiol. Rev.* 56: 465-501.
- STEIN, P. S. G. 1978 Motor systems, with specific reference to locomotion. *Ann. Rev. Neurosci.* 1: 61-82.
- WALLÉN, P. 1977 On the spinal generation of locomotion in fish. In: *Neurophysiological mechanisms of locomotion. Satellite Symposium*. 27th Internat. Congr. of Physiol. Sciences, Paris.
- WICKELGREN, W. O. 1977 Physiological and anatomical characteristics of reticulospinal neurons in lamprey. *J. Physiol.* 270: 89-114.

Existence of an avian pancreatic polypeptide (APP) immunoreactive substance and catecholamines in some peripheral and central neurons

by LUNDBERG TOMAS HÖKFELT ANDERS ÅNGGÅRD JOE KIMMEL,
T. KOOLDSTEIN and KENNETH MARKEY

From the Department of Histology, Karolinska Institute and Department of Oto-Rhino-Laryngology, Karolinska Hospital, S-141 86 Stockholm, Sweden; The University of Kansas Medical Center, Kansas and Department of Surgery, New York University Medical Center, New York, NY, USA

Catechol polypeptide (PP) was isolated by Kimmel et al. (1971, 1975) from chicken pancreas and initial characterization of avian PP (APP) revealed that it is built up from 36 amino acids (see Kimmel et al. 1975). Recently Lörén et al. (1979) described a wide distribution of APP immunoreactive neurons in both the central and peripheral nervous system.

Endocrine cells in the gastro-intestinal tract and peripheral tissues often contain both a catecholamine and a polypeptide hormone (see Ånggård & Polak 1978) and this has recently been observed also in neurons (see Hökfelt et al. 1980). In search for further examples of coexistence situations we have examined in detail the distribution of APP in relation to some known transmitter related substances. In the present preliminary paper we report a peptide reacting with the APP antiserum can be observed in sub-populations of several types of catecholamine (CA) neurons in rat and cat.

Antibodies to APP were raised in rabbits (Kimmel et al. 1975). The antiserum was used in dilution of 1:100. APP from salmon was used as control. For detection of antibodies to tyrosine hydroxylase (TH) and catecholamine N-methyltransferase (PNMT), two catecholamine synthesizing enzymes purified from the rat nucleus accumbens and rat adrenal glands, respectively (Markey et al. 1980) and Goldstein et al. (1978). Male rats (Sprague Dawley, b.w.t. 150-200 g) and adult cats of both sexes were used. Two rats received an intracerebral injection of colchicine (60 µg in 20 µl). Two received two injections of 6-hydroxydopamine (6-OH-DA) (100 mg/kg) 48 h and 24 h before sacrifice. In 2 the sciatic nerve was ligated 24 h before sacrifice. In 3 the superior cervical ganglion was extirpated 14 days before sacrifice. The experimental animals were perfused with 10% ice-cold formalin or fixed with modified Bouin's (Lundberg, in preparation) and the brains, spinal

cord and some peripheral tissues were dissected out. After further fixation by immersion and rinsing, consecutive cryostat sections were processed for the indirect immunofluorescence procedure. Briefly 3 consecutive sections were incubated with antibodies to TH, APP and PNMT respectively at +4°C for 24 h, rinsed, incubated with fluorescein isothiocyanate (FITC) conjugated sheep anti-rabbit antibodies for 30 min at +37°C, rinsed, mounted and examined in Zeiss fluorescence microscope. In some experiments the elution and re-staining technique of Tronzo et al. (1978) was used to establish coexistence of two antigens in the same cell bodies.

In the peripheral nervous system of the rat APP like immunoreactivity was observed in the majority of the principal sympathetic ganglion cells in the superior cervical, the stellate and the celiac ganglia. APP immunoreactive nerve terminals were widely distributed. Thus APP immunoreactive plexuses were seen in high concentrations in the vas deferens (Fig. 1A) and in the heart, with particularly high amounts in the arteries. In the submaxillary gland and the iris only few fibres were observed. On adjacent sections the APP-immunoreactive cell bodies were also positive to TH (but not to PNMT) in the ganglia described above. Also nerve fibres containing TH were seen in the submaxillary gland, heart and vas deferens (Fig. 1B) with a similar distribution pattern. However, so far many more TH than APP immunoreactive fibres were seen in the iris. Furthermore, in the submaxillary gland the APP positive fibres were mainly seen in relation to blood vessels whereas the fibres containing TH in addition were seen in the exocrine parenchyma. After 6-OH-DA treatment a marked decrease in the number of both APP and TH immunoreactive fibres was observed in the submaxillary gland, heart and vas deferens (Fig. 1C). After ligation of the sciatic

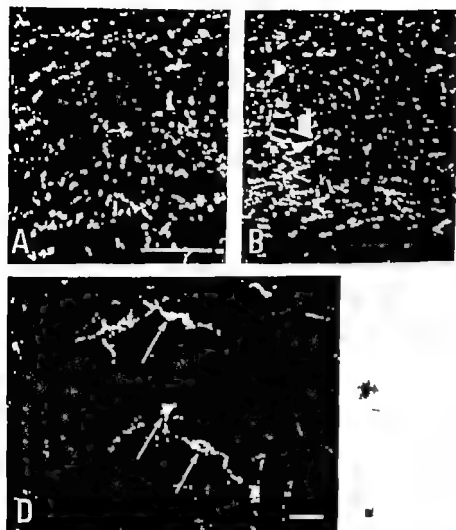


Fig. 1 A-F Immunofluorescence micrographs of the vas deferens (A-C) and ventrolateral medulla (D-F) of untreated (A, B), 6-hydroxydopamine (6-OH DA) (C) and tyrosine hydroxylase (TH) (B, F) and F show the same section (elution according to Lundberg et al. 1978). (A-C) Dense network of APP immunoreactive and catecholamine nerve terminals are seen in the vas deferens. After 6-OH DA treatment most of the APP and TH (not shown) immunoreactive fibres disappear. Neuronal cell bodies (arrows) contain both APP-like immunoreactivity and TH. Bars indicate 50 μ m.

nerve a marked accumulation of both APP and TH like immunoreactivity was observed mainly central to the ligature. The accumulation of immunoreactivity exhibited a marked overlap when comparing the adjacent sections incubated with the respective antibody.

In the *cat* peripheral nervous system the majority of neuronal cell bodies in the cervical stellate and celiac ganglia were both APP and TH immunoreactive. No certain APP like immunoreactivity could be seen in the sphenopalatine ganglion cells which belong to the parasympathetic system. APP immunoreactive fibres were found in most peripheral tissues including heart, particularly auricles, nasal mucosa, vas deferens and in low amounts in the

submandibular gland and iris. TH immunoreactive fibres were seen on adjacent sections of the iris with a rather similar distribution. Removal of the superior cervical ganglion and TH immunoreactive fibres in the nasal mucosa completely disappeared. Both in the *cat* and *rat* APP like immunoreactivity was observed in a population of adrenal medullary gland cells.

In the *rat* central nervous system APP immunoreactive neuronal cell bodies and peripheral exhibited a wide spread distribution as partly described by Lörén et al. (1979). Here some of the system will be dealt with, i.e. containing both the peptide and catecholamine synthesizing enzyme. Elution and retaining

Tramu et al (1978) revealed that cell immunoreactive to both APP (Fig. 1 D) TH (1) and PNMT with identical distribution were observed mainly in two groups in the oblongata: (1) the nucleus tractus solitarius extending dorsally into the midline area of sulcus longitudinalis medialis and (2) the lateral aspects of the medulla oblongata, rostrum between the nucleus facialis and the nucleus tegmenti and caudally in the ventral parts of the reticular nucleus. A few APP and TH (1) PNMT immunoreactive cells were also in the area caudal to the area postrema, and in the ventral part of the locus coeruleus. APP TH (1) PNMT immunoreactive nerve terminals with specific distribution patterns were observed in the sympathetic lateral column of the spinal cord, tractus solitarius, locus coeruleus, nucleus striatus lateralis, several hypothalamic nuclei with particularly high concentration in the infundibular area. Results on the rat central nervous system will not be reported here. Absorption with abolished the immunostaining in both peripheral and central areas.

Present findings give strong evidence that a class of peripheral noradrenergic sympathetic innervating heart, vas deferens and other lateral tissues contains an APP-like peptide which is axotically transported. This evidence is based on (1) the identity of APP and TH immunoreactive cell bodies in sympathetic ganglia, (2) distribution patterns for terminals and axons reactive to these antisera, (3) disappearance of APP and TH immunoreactive fibres in lateral trunks after 6-OH DA treatment and (4) presence of such fibres in the nasal mucosa, sympathetic innervation of the central nervous system the APP-like peptide is present in a population of adrenaline cell bodies belonging to the C1 and C2 groups of Hökfelt et al (1974). Furthermore overlapping distribution patterns of APP and PNMT TH immunoreactive nerve terminals were in many areas of the brain and spinal cord (cf. Tramu et al 1974). In addition APP was present in noradrenergic neurons of the locus coeruleus. The A1 group of the lower medulla oblongata and present findings add a further example of

coexistence of a peptide and a monoamine transmitter in neurons (see Hökfelt et al 1980). The identity of the APP-like immunoreactivity is not known and it will be an important task to elucidate its genuine structure. The distribution of the APP-like peptide suggests a possible role i.e. in the regulation of hemodynamic events.

The present study was supported by grants from the Swedish Medical Research Council (04X 2387, 17X 5438), Magnus Bergvall Stiftelse and Årshjort Alice Wallenberg's Stiftelse.

We are grateful to M. J. Backman, M. V. Hort, M. U. Landefeldt and M. G. Norell for skilful technical assistance.

REFERENCES

- GOLDSTEIN M, LEW J Y, MATSUMOTO Y, HÖKFELT T & FUXE, K 1978. Localization and function of PNMT in the central nervous system. In Psychopharmacology: A generation of progress (ed M. A. Lipton, A. DiMascio & K. F. Williams), pp 361-369. Raven Press, New York.
- HÖKFELT T, FUXE, K, GOLDSTEIN M & JOHANSSON O 1974. Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. *Brain Res* 86, 33-35.
- HÖKFELT T, JOHANSSON O, LUNDQVIST, A, LUNDBERG M J & SCHULTZBERG M 1980. Peptidergic neurons. *Nature (Lond)* 284, 515-517.
- KIMMEL, J R, HAYDEN L J & POLLOCK H O 1975. Isolation and characterization of a pancreatic polypeptide hormone. *J Biol Chem* 250, 9369-9376.
- LOREN I, ALUMETS, J, HÄKANSSON M & SUNDLER, F 1979. Immunoreactive pancreatic polypeptide (PP) occurs in the central and peripheral nervous system. Preliminary immunocytochemical observations. *Histochemistry* 78, 179-186.
- MARNEY K, A, KONDO S, SHENKMAN L & GOLDSTEIN M 1980. Purification and characterization of (tyrosine hydroxylase) from a clonal pheochromocytoma cell line. *Mol Pharmacol* 17, 79-85.
- PEARSE, A G E & POLAK J M 1978. The diffuse neuroendocrine system and the APUD concept. In Gut hormones (ed S. R. Bloom), pp 31-79. Churchill Livingstone, Edinburgh.
- TRAMU G, PILLEZ, A & LEONARDELLI J 1978. An efficient method of antibody elution for the successive or simultaneous location of two antigens by immunocytochemistry. *J Histochem Cytochem* 26, 322-324.

We have added in proof Tateishi and Mattioli recently isolated PP-like peptide from mammalian intestine and brain (*Nature* 1980, 285, 417).

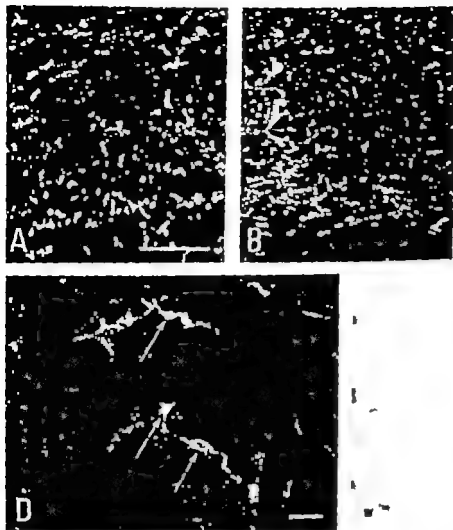


Fig. 1 A-E Immunofluorescence micrographs of the vas deferens (A-C) and ventrolateral medulla oblongata of untreated (A-B) 6-hydroxydopamine (6-OH DA) (C) and colchicine (D-E) treated rat after incubation with anti-APP (A-C) and tyrosine hydroxylase (TH) (B-E). D and E show the same section (elution according to Lundberg et al. 1978). (A-C) Dense network of APP immunoreactive and catecholamine nerve terminals are seen in the vas deferens. After 6-OH DA treatment most of the APP and TH (not shown) immunoreactive fibers disappear. Neuronal cell bodies (arrows) contain both APP-like immunoreactivity and TH. Bars indicate 50 μ m.

nerve a marked accumulation of both APP and TH like immunoreactivity was observed mainly central to the ligature. The accumulations of immunoreactivity exhibited a marked overlap when comparing the adjacent sections incubated with the respective antibody.

In the cat peripheral nervous system the majority of neuronal cell bodies in the cervical stellate and celiac ganglia were both APP and TH immunoreactive. No certain APP like immunoreactivity could be seen in the sphenopalatine ganglion cells which belong to the parasympathetic system. APP immunoreactive fibres were found in most peripheral tissues including heart, particularly auncles, nasal mucosa, vas deferens and in low amounts in the

submandibular gland and iris. TH immunoreactive fibres were seen on adjacent sections of the tissue with a rather similar distribution. After removal of the superior cervical ganglion, the APP and TH immunoreactive fibres in the nasal mucosa completely disappeared. Both in the cat and rat APP like immunoreactivity was observed in a population of adrenal medullary gland cells.

In the rat central nervous system APP immunoreactive neuronal cell bodies and nerve terminals exhibited a wide spread distribution as partly described by Lorén et al. (1979). Here some of the systems will be dealt with, i.e. those containing both this peptide and catecholamine synthesizing enzymes. Elution and reanalysis

influence of circulating adrenaline on extracellular potassium concentration in the brain

LIGRINO and B. K. SIESJO

From the Experimental Brain Research E-Blocklet, University Hospital Lund, Sweden

Circulating adrenaline gains access to brain by an abrupt elevation of arterial blood flow. Both cerebral blood flow (CBF) and rate of oxygen (CMR_{O_2}) are markedly reduced (Bernstein et al. 1978; Dahlgren et al. 1978). Probably this catecholamine effect is due to its effect on cerebral metabolism, with the fall in CBF following secondarily (cf. Macdonald et al. 1976). However studies to date have demonstrated any reasonable link between the changes in CBF and CMR_{O_2} , nor have they disclosed the mechanism(s) causing the rise in CMR_{O_2} . These studies have failed to reveal significant changes in tissue concentrations of glycogen, lactic acid, AMP or signs of neuronal activation (Siesjö et al. 1980). Increases in CMR_{O_2} and CBF of similar magnitude are found during seizures, then accompanied by an elevated concentration of extracellular potassium concentration. For experimental results and further literature (Astrup et al. 1979). It is therefore tempting to speculate that adrenaline enhances CMR_{O_2} by releasing K^+ from cells and that this variable is also responsible for the increase in CBF. The present study was undertaken to investigate the effect of adrenaline on extracellular fluid $[K^+]_e$ in the cerebral cortex. In order to eliminate the possibility of any direct effect on $[K^+]_e$ of the increase in pressure, we have also included a series of experiments in which arterial pressure was increased in the absence of added adrenaline.

Male Wistar rats (300–450 g) were used. Anaesthesia was induced with 3% halothane and N_2O . During operative procedures halothane was reduced to 1%. Following surgery the animals were maintained on 70% N_2O and 30% O_2 . They were tracheotomized, immobilized with urethane chloride ($1.5 \text{ mg kg}^{-1} \text{ i.v.}$) and connected to a respirator. In the adrenaline-infused group, femoral arterial and venous catheters were

inserted. In the other group, catheters were placed in the brachial arteries (see below). This allowed us to monitor blood pressure, take samples for blood gas and pH analysis and to infuse drugs. Following catheter insertion, the skull was exposed and a craniotomy was made on one side. The head was then immobilized and a double-barrelled K^+ microelectrode (tip diameter 1–2 μm) was inserted through a small slit in the dura. The construction and use of the K^+ electrode has been previously described (Astrup & Norberg 1976). The animal was then placed in a Faraday cage. Rectal temperature was maintained close to 37°C through the use of an infrared heating lamp. All animals were maintained under normoxic ($PaO_2 > 100 \text{ mmHg}$) normocapnic ($PaCO_2 34\text{--}40 \text{ mmHg}$) conditions. Two groups of animals were studied. In one group ($n=18$) adrenaline ($8 \mu\text{g kg}^{-1} \text{ min}^{-1}$) was infused i.v. over a period of 10 min. In the other group ($n=5$) in order to induce a rise in arterial pressure the abdominal aorta was clamped and the clamp left in place over a period of 10 min. In both groups the K^+ potential was continuously monitored before, during and following either adrenaline infusion or aortic clamping. Statistical differences within groups were calculated using the paired Student's *t*-test.

Results and discussion In both groups body temperature was kept close to 37°C. Blood gases remained at the levels mentioned above. Arterial blood pressure changes were strikingly similar between the two groups (Fig. 1 upper part). This allowed us to distinguish any independent effect of an increased systemic blood pressure on brain extracellular fluid $[K^+]_e$.

Fig. 1 (lower part) shows the changes in $[K^+]_e$ during 10 min of adrenaline infusion and 10 min of increased arterial pressure via clamping of the abdominal aorta. This figure clearly shows that a rise in arterial pressure, by itself, has no effect on $[K^+]_e$.

Juxtaglomerular cell activity during hemorrhage and ischemia as revealed by quinacrine fluorescence

ALUND M. Department of Histology, Karolinska Institute and Department of General Surgery, Karolinska Hospital, Stockholm, Sweden

ALUND M. Juxtaglomerular cell activity during hemorrhage and ischemia as revealed by quinacrine histofluorescence. *Acta Physiol Scand* 1980; 110: 113-121. Received 4 Dec. 1979. ISSN 0001-6772. Department of Histology, Karolinska Institutet and Department of General Surgery, Karolinska Hospital, Stockholm, Sweden.

Quinacrine (QC) binds with high affinity to the intracellular storage granules of juxtaglomerular cells (JG-cells) in the afferent arterioles of the glomerulus of the kidney. The present study tests whether QC bound to JG-cells can be released. The cells were stimulated by renal ischemia and hemorrhagic shock combined with immobilization stress. 1 h after onset of renal ischemia QC JGI (modified Hartroft & Hartroft 1953) in ¹⁴C-QC treated rats had decreased to about 40% in the ischemic kidney compared to not ligated control kidney. The ¹⁴C-contents in the ischemic kidney had decreased to 33% of that in the nonligated control kidney. Hemorrhagic shock was obtained by bleeding into reservoir for 15 min or 1 h. Rats who received QC or ¹⁴C-QC 1 h before onset of bleeding showed no change in QC JGI (15 min shock) or ¹⁴C-contents (1 h shock) as compared to controls. This was probably due to formation of new QC-binding granules.

Which took up still circulating quinacrine thereby masking release. If the time between the QC injection and the onset of shock was extended to about 15 h when circulating amounts of QC are very low, decrease of QC JGI (about 30% of controls) was seen in the kidneys of the shocked rats. The results are compatible with the possibility that QC in vivo bound to granules of JG-cells could be released together with the content of the granules following stimuli known to induce renin release. Quinacrine-binding therefore possibly provides a new method to study endocrine cells in the way it has been used in the present study as marker of JG-cell activity.

Key words: Quinacrine histofluorescence-juxtaglomerular cells-renal ischemia-hemorrhagic shock.

Quinacrine (QC) is a fluorescent acridine derivative which selectively binds to nerve fibres and cells in the gut and elsewhere (Olsson et al. 1976, 1978, Ålund & Olsson 1978, 1979; Ålund & Olsson 1978; Ålund & Olsson 1979; Olsson & Ålund 1978; Ålund & Olsson 1978, 1979). This binding seems to be closely linked to an release mechanism (Ålund & Olsson 1978).

QC bound to nerve fibres is released by stimulation (Ålund & Olsson 1978, 1979). Recently it is reported (Ålund & Olsson 1979b) that QC has a high affinity to hormone storing cells of endocrine cell containing dense core granules (Fig. 1b). Since such cells release hor-

mones from their storage granules it was of interest to know whether QC, presumably bound to the same vesicles (Fig. 1b), could be released with the hormone or not.

Renin-release from the juxtaglomerular cells (JG-cells) of the afferent arterioles of the glomerulus in the kidney has been thoroughly studied especially at renal hemodynamic disturbances. Although not endocrine cells in the classical sense JG-cells were chosen as a model for release activity. Renal ischemia and hemorrhagic shock were used to induce release from the cells. The results indicate that QC bound to storage granules of the

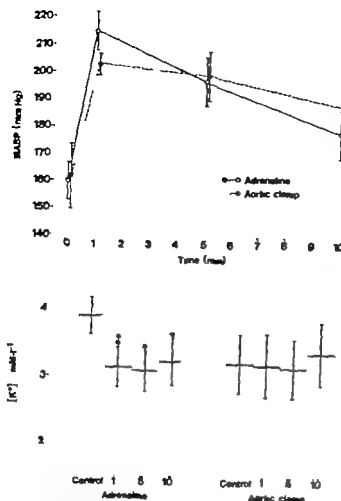


Fig. 1 The influence of adrenaline and clamping of the abdominal aorta on arterial pressure (MABP) (upper part) and brain extracellular fluid $[K^+]_o$ (lower part) over time. Values are given as mean \pm S.E. $P < 0.001$ and $P < 0.01$.

However, adrenaline causes a reduction in $[K^+]_o$ to about 80% of normal. The reductions were all highly significant when comparing the $[K^+]_o$ at 1 ($P < 0.001$), 5 ($P < 0.001$) and 10 min ($P < 0.01$) of adrenaline infusion to the $[K^+]_o$ prior to infusion.

The results demonstrate that the adrenaline-induced increase in CMR_i cannot have been due to an elevation of $[K^+]_o$, as due to an enhanced release from cells. Rather, it seems likely that the adrenaline-induced reduction in $[K^+]_o$ was the result of an increased Na^+K^+ pump activity (see Phillis 1974). Possibly the increase in CMR_i with adrenaline is somehow related to this enhanced pump activity. Yet the present results do not allow us to distinguish whether this is a cause-effect relationship or merely parallel phenomena. However, results from the present study indicate that extracellular fluid $[K^+]_o$ is not the link between the in-

creased CMR_i and increased CBF measured in the enhanced tran location of adrenaline at blood-brain barrier at least not according to the classical concept of $[K^+]_o$ acting as a vasodilator. Obviously the link between increased CMR_i and blood flow still remains to be elucidated.

This study was supported by grants from the Medical Research Council (project No. 1075) and USPHS (No. 5R01 NS07330). D. Pellegriano is an NIH post-doctoral fellow.

REFERENCES

- ASTRUP J & NORBERG K 1976. Effects of hypoxia on cerebral cortex in rats during propofol anesthesia. *Brain Res* 102, 418-421.
- ASTRUP J, BLENNOW G & NILSSON L 1976. Effects of reduced cerebral blood flow on short-term cerebral extracellular potassium and metabolism in the rat cortex during induced seizures. *Brain Res* 177, 115-124.
- BERNTMAN L, DAHLGREN N & SIESJÖ B 1978. Influence of intravenously administered cholinergics on cerebral oxygen consumption and blood flow in the rat. *Acta Physiol Scand* 110, 145-154.
- DAHLGREN N, ROSEN I, SAKABETI A & SIESJÖ B 1980. Cerebral functional metabolic effects of intravenous infusion of adrenaline in the rat. *Brain Res* 184, 143-154.
- MACKENZIE E, T. McCULLOUGH J, J. M. PICKARD J D & HARPER, A. M. 1974. Cerebral circulation and noradrenaline. Release of blood-brain barrier. *Am J Physiol* 231, 17-21.
- PHILLIS J W 1974. The role of calcium in the effects of biogenic amines. *Life Sci* 14, 119-124.

of control rats

young male rats were injected with QC 1 mg/kg 1 h before sacrifice (3 rats) after QC was given their kidneys were removed via the abdomen during ether anaesthesia and for QC-JG estimation (see next paragraph)

for microscopy and juxtaglomerular cell index

Various time-lapses and varied experimental procedures below) kidneys were rapidly dissected out of ether-anaesthetized animal. The kidneys were along their lateral convex border freeze-dried (Ungerstedt 1970) and paraffin-embedded.

Paraffin-embedded kidneys were sectioned at 8 μ m so that the entire cortical area of the cut of the renal half was covered in one section. Several coded sections were visually examined and the representative whole cut surface was selected for fluorescence microscopy and estimation. Specimens examined in dark-field (fluorescence microscope) and for Falck-Hillarp fluorescence histochemistry (Falck & Johansson 1967; Falck *et al.* 1962). A light microscopical method to evaluate granule content in JG-cells described by Hartroft & Hartroft (1953) who used specific granule stain according to Watson (1952). The content of granule content in every sample JG-apparatus in one section was made on a serial quantitation scale with 4 steps (1-4). Step 1 represented apparatuses with cells containing few granules scattered in the nucleus. Step 2 meant one to three cells granules spread throughout the cytoplasm but completely filling it. Step 3 meant 1-3 cells with the area filled with densely packed granules or many of the second category. Step 4 meant several cells of 3 category. The totals recorded under steps 1, 2, 3 were multiplied by the factors 1, 4 and 8 respectively, at very conservative estimate at least 8 is many granules were present in groups of cells at step 4, as were present in those classified as (Hartroft & Hartroft 1953). Such weighted totals expressed per 100 glomeruli to obtain juxtaglomerular index (JGI).

QC binds to and marks the storage-vesicles of cells (Fig. 1b; Akmal & Olsson 1979) the same principle method to estimate granule content as was used in the fluorescence microscope to obtain the juxtaglomerular granulation index (QC-JGI). Microscopy specimens were coded and all evaluations made blindly by the same investigator. All estimates on complete section from each kidney counted and evaluated.

Formula for QC-JGI as follows

$$\frac{1 \times b + 4 \times d}{N_{\text{glom}}} \times 100$$

1 = number of JG-apparatuses (in the complete section)

b = QC-fluorescent granule content estimated as step 1

d = QC-fluorescent granule content estimated as step 3

N_{glom} = number of JG-apparatuses (in the complete section)

QC-fluorescent granule content estimated as step 3

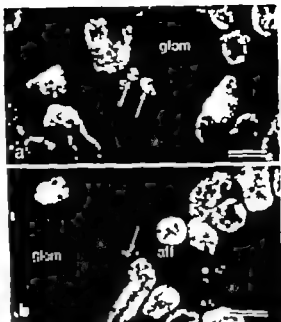


Fig. 2. (a) Untouched kidney of rat 1 h after single QC-injection of 1 mg/kg. The cytoplasm of the juxtaglomerular cells (arrows) is densely packed with QC-fluorescent granules. The glomerulus (glom) is not fluorescent. Tubular parts of the nephron are unspecifically auto-fluorescent. Note that cell nuclei are not fluorescent. Fluorescence microphotograph of 8 μ m section, marker 50 μ m. (b) The one hour ischemic kidney of the same rat as in (a). The cytoplasm of the juxtaglomerular cells (arrow) in the afferent arterioles (aff) contains only few QC-positive granules. The glomerulus (glom) is non-fluorescent while tubular parts of the nephron are unspecifically auto-fluorescent. Fluorescence microphotograph of 8 μ m section, marker 50 μ m.

d = number of JG-apparatuses (in the complete section) with QC-fluorescent granule content estimated as step 4

N_{glom} = total number of glomeruli found in the complete section.

Theoretically the QC-JGI could vary within the limits 0 to 800 (800 when a, b and d are 0 and d equal N_{glom}).

Quantitative measurement of ¹⁴C-quanacrine content in kidneys

From the kidneys of animals that had received ¹⁴C-QC punches of about 10 mm² were taken from cortical areas just beneath the surface of the kidney. They were taken from the halves of the kidneys that were not used for fluorescence microscopy. The punches were weighed (Cahn electrobalance), dissolved in solution (Packard) and radioactivity measured by liquid scintillation (Packard Tri Carb 3002). Counts per minute per mg wet tissue were calculated. ¹⁴C-QC (spec. act. 16 mCi/mmol) was generously gift from Hoffmann-La Roche Ltd, Switzerland.

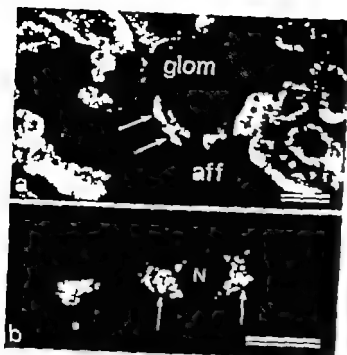


Fig. 1 (a) Kidney of rat 1 h after QC i.v. 1 mg/kg. Granules in the juxtaglomerular cells are intensely fluorescent (arrows) and therefore somewhat overexposed. No fluorescence is found in the glomerulus (glom) or the afferent arteriole (aff). Tubular parts of the nephron are unspecifically autofluorescent. Fluorescence microphotograph of 8 μ m section marker 50 μ m. (b) Close up on one JG-cell in the kidney of a rat 1 h before sacrifice received QC 1 mg/kg i.v. The cytoplasm is filled with 25–30 intensely fluorescent densely packed granules (arrows). No fluorescence at all is found in the cell nucleus (N). To the left a minor part of a second JG-cell can be seen. Fluorescence microphotograph of 6 μ m section marker 10 μ m.

JG-cells (Fig. 1) is released when renin release occurs. QC can thus be used as a histochemical marker of juxtaglomerular cell activity.

MATERIAL AND METHODS

56 male albino rats (Sprague-Dawley 250 g b.w.) were used. During a short period of their anesthesia they were injected intravenously in the sublingual vein with quinacrine hydrochloride (Sigma) or 3 C-labelled quinacrine hydrochloride (3 C-QC, Hoffmann-La Roche, Switzerland) dissolved in saline. The injected dose (QC as well as 3 C-QC) was 1 mg/kg given in a volume of approximately 0.3 ml.

Renal artery ligation

1 h after 3 C-QC the right or left renal artery was exposed (either anesthesia) by a translumbar retroperitoneal approach and ligated in 8 rats. They were then allowed to wake up. 1 h after ligation both kidneys were dissected out via an abdominal incision and ether-anesthesia. Punches for 3 C-QC content measurement were taken

from both kidneys. The remaining part of the freeze-dried and paraffin-embedded. Not ligated were used as controls (untouched side).

Hemorrhagic shock

The 34 rats were given water ad libitum and fed a diet which was withheld about 18 h before they or 1–20 h before shock was started experimentally. received QC or 3 C-QC i.v. The time between injection and shock will be referred to as t below. Every experimental animal with a QC i.v. and a subsequent bleeding time of different length corresponding control animal with an equal length between QC injection and kidney dissection. In the experimental design the 16 rats with longer QC t 1 h did not all have a QC time of equal length. For experimental rats QC-time plus the bleeding time kidney dissection ranged from 1 to 20 h (mean 11 h). For the 7 control rats the time between QC and kidney dissection ranged from 12 to 24 h (15.6 \pm 0.9 h). The experimental procedure was performed according to Whigham & Weil (1966) with modifications (Farnebo & Hamberger 1977). The method established and will only be briefly described for animals were anesthetized with ether during the procedure (connecting animals to remanent pressure transducer). Experimental rats were immobilized by being wrapped in metal nets (Kron 1971) and allowed to wake up and recover for before the onset of bleeding. They were bled in favor to a systolic pressure of 70 mmHg measured a heparinized femoral artery catheter by a blood pressure transducer (Statham P23 AC) connected to a Gough graph. When they had had a stable low pressure for 1 h kidneys were dissected out (ether-anesthesia), freeze-dried, paraffin-embedded and sectioned (approximately 10 μ m). They were taken from cervical 3 C-QC activity measurement. Controls were anesthetized by ether at an equal length of time as experimental rats. In order to increase differences in renal control were not shamoperated or immobilized.

Calculation from plasma after 3 C-QC

During ether anesthesia heparinized catheters were inserted in the right femoral artery of 4 rats. Each bled for 8 h and heparin 300 U/kg i.v. from Sweden injected. They were then given 1 mg/kg 3 C-QC i.v. and immobilized by being wrapped in metal nets (Corrodi et al 1971). Blood samples (0.25 ml) were collected after 30 and 170 min (rats) or after 40 min (rat). The 3 C-QC measured from the femoral catheters given i.v. After 4 h the metal nets (all 4 rats) femoral catheters were removed and animals were left. 4 h after the QC injections blood samples collected from the left ventricle of the heart. Blood samples were immediately centrifuged (6000 g 10 min) and 100 μ l plasma was dissolved in Soluene (Packard) counted by liquid scintillation. Using 3 C toluene standard the disintegrations per minute per μ l plasma were calculated (dpm/ μ l plasma).

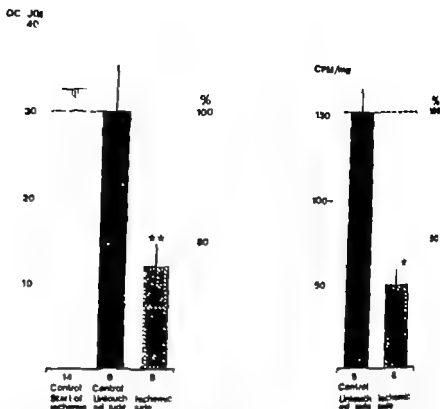


Fig. 3 (a) Diagram to the left. Effects of renal ischemia (1 h; bar to the right) on QC JGI values of animals which 1 h before ischemia received ^{14}C -QC 1 mg/kg i.v. A statistically significant ($P \leq 0.01$) reduction of QC JGI compared to the untouched kidney (middle bar) is found. Both these bars represent the average of 4 right and 4 left kidneys. The left bar represents QC JGI values found in 7 rats (7 right kidneys and 7 left kidneys; $n=14$) 1 h after QC injection of 1 mg/kg. (b) Diagram to the right. Effects of 1 h renal ischemia on cpm/mg wet weight in animals which 1 h before ischemia received ^{14}C -QC 1 mg/kg i.v. A statistically significant ($P \leq 0.001$) decrease is seen on the ischemic side compared to the untouched side.

Statistical analysis

For statistical analysis individual estimations were ranked and tested with the Mann-Whitney non-parametrical test. Mean values \pm S.E.M. were used throughout this paper.

RESULTS

Fluorescence features and QC JGI for controls

The 7 rats that were injected i.v. with 1 mg/kg QC showed a very intense QC fluorescence in the granules of the juxtaglomerular apparatuses in the afferent arterioles 1 h later (Fig. 1). In some JG cells most granules were located around the nucleus. In others the entire cytoplasm was filled with densely packed granules. If QC was given 1–20 h before sacrifice the fluorescence was still quite strong but clearly weaker than after 1 h. The QC fluorescence intensity in the granules was subjecti-

vely estimated to about 30 or 40% of the intensity after 1 h. In addition to the QC fluorescence, which is bright green, the kidneys contained an autofluorescence. This was located in the proximal parts of the nephron (Fig. 1a and b), in the proximal tubule. It was first described by (I. Sjöstrand *thesis*). The autofluorescence had a green yellow or brown color and therefore was not distinguished by fluorescence microscope. QC JGI for those 7 control animals which had QC 1 h before sacrifice were found to be 3.6 ± 0.4 left and right kidneys (Fig. 3a left bar). QC values for the 3 control animals that were sacrificed 15 h after a QC injection of 1 mg/kg were 6.1 ± 0.3 ($n=6$ left and right kidneys). No systematic difference between left and right kidneys was found.

tens ligation

existence in granules of the untouched kidney quite normal both concerning fluorescence intensity and granule content (Fig. 3a). In the

kidney granular fluorescence intensity unchanged but the granule content appeared to be reduced (Fig. 2b). The untouched had QC JGI of 30.3 ± 5.3 while the ischemic had decreased QC JGI of 12.1 ± 2.6 or the control value (Fig. 3). The difference significant ($P < 0.01$). The ischemic kidney contains some 33% of the ^{14}C -counts/mg wet tissue in the untouched kidneys (Fig. 3b). The difference is significant ($P < 0.001$). Separate values for left and right kidneys are given in Table 1.

Correlations between individual QC JGI and total ^{14}C measurements were positive ($r = 0.70$). The correlation between group mean QC JGI and group mean ^{14}C -QC contents given in Table 1 was also positive ($r = 0.92$, $n = 4$).

hemorrhagic shock

Fluorescence intensity of individual granules after hemorrhagic shock was not affected as compared to the control animals. The granules seemed normal and had a normal location within the cell. In the experiment with long QC-time fluorescence intensity had decreased as much in controls as in experimental animals.

Shock 15 min QC-time 1 h Bleeding volume in reservoir at the end of the shock period ranged from 8 to 3.2% of the body weight. QC JGI for experimental rats and the 3 control rats is given in Fig. 4. None of the QC JGI mean differences were significant.

Shock 1 h QC-time 1 h In this experiment ^{14}C was used. The average bleeding volume in the reservoir at the end of the shock period was 1.6 to 3.4% of the body weight. ^{14}C contents of kidneys in

Effect of chemical on quinacrine content in juxtaglomerular cells. Same animals as in fig. 3

	Ischemic		Untouched	
	QC JGI	^{14}C -QC	QC JGI	^{14}C -QC
Left	7.2 \pm 9.53	18	39.3 \pm 5.7	179 \pm 9
Right	14.3 \pm 3.14	43	21.3 \pm 6.6	125 \pm 16



Fig. 4 (a) Diagram to the left. Bars representing QC JGI values of left and right kidneys of control animals and 15 min shock animals (1 h QC-time). No difference between shock and control group is revealed. (b) Diagram to the right kidneys of control animals and 1 h shock animals (1 h QC-time). No differences between shock and control group is revealed.

cpm/mg wet tissue are given in Fig. 4b. No difference between experimental and control rats was found.

Shock 1 h QC-time 12-20 h Average bleeding volume in the reservoir at the end of the shock period was 2.7 to 3.4% of the body weight. Both for

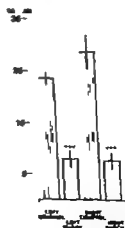


Fig. 5 Effects on QC-JGI in right and left kidneys of animals with long QC-time (15.6 h on average). A statistically significant ($P < 0.001$) of QC JGI is seen after 1 h of hemorrhagic shock.

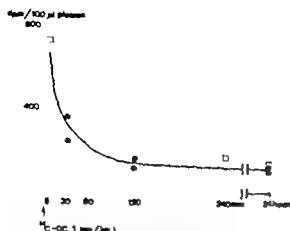


Fig. 6 The elimination of ^{14}C from plasma in 4 rats (each symbol represents one rat) after injection of ^{14}C -QC 1 mg/kg. Rats were immobilized from the ^{14}C -QC injection to minute 740. dpm = disintegrations per min.

right and left kidneys a strongly significant reduction of QC JGI was found in the shocked animals ($P < 0.001$) (Fig. 5). The decrease in granularity corresponds to 34.5% (left kidney) and 77.8% (right kidney) of corresponding control side.

The difference between the left and right kidney was not significant.

^{14}C -elimination from plasma after ^{14}C -QC injection

4 rats were injected with ^{14}C -QC and elimination of ^{14}C from the plasma was studied. The results are shown in Fig. 6. Elimination of ^{14}C from plasma was initially rapid. After 30–60 min about 50% of the activity at 5 min remains. A steady state level seems to be reached after 120–240 min and thereafter the elimination over time occurs to be very slow.

DISCUSSION

QC in vivo has a strong affinity to endocrine cells with large dense core vesicles (Ålund & Olson 1979b). Since QC bound in vitro to nerve fibres in Auerbach's plexus (Olson et al. 1976) can be released by depolarization in an at least partly Ca^{2+} dependent manner (Ålund & Olson 1978, 1979a). It was interesting to see if QC in vivo bound to endocrine cells could be released by endocrine activity of the cells. Although renin is an enzyme and not a hormone in the classical sense it is probably stored

in the JG granules in a manner similar to that of polypeptide hormones in large dense core vesicles. For several reasons the JG-cells of the arterioles in the kidney were chosen as a release activity. First the rather large population of these cells contain renin (Edelman & Hix, 1974) and in vivo bind QC (Fig. 1b). Ålund & Olson (1979a) have used for QC fluorescence microscopy have devised a method to estimate the number of JG-cells which with slight modification can be used for QC fluorescence microscopy providing a possibility to quantify QC in granules in these cells. The validity of these estimations is illustrated by the positive correlation between radioactivity and QC JGI in the kidney with renal artery ligation. Third, the JG-cells which trigger renin release in particular hemodynamic disturbances of the kidney are known. Ever since Goldblatt et al. (1934) demonstrated that renal artery constriction produced hypertension in the dog it has been established that renal ischemia is a primary stimulus for renal renin release. Convincing evidence for the presence of an intrarenal vascular receptor to renin release was provided by Blaine et al. (1971a, b). Stimulation of renal nerves also results in an increased renal renin release (Lambert, Johnson et al. 1971) mediated via sympathetic activity. A β -receptor may trigger the renin-output (Winer et al. 1969; Assouline, 1970). Also circulating catecholamines influence renin secretion (Johnson, David & Winer, 1970). Chronic sodium depletion, various hormonal decreased sodium or chloride load at the distal tubule also influence renin release (for review see Davis & Freeman 1976 and Reid et al. 1977).

To obtain a strong patho-physiological stimulus for renin secretion severe bleeding control immobilization stress was used. In addition the renal artery was used in some experiments to obtain maximal albeit non-physiological release of JG-granules. Both these probably influence JG-cells via one or several of the above reviewed mechanisms. 1 h after ligation of the renal artery the QC in the ligated side decreased to about 40% ($P < 0.001$) of the untouched control side. The radioactivity in the untouched control side was reduced to about 33% ($P < 0.001$) of the control side (Fig. 3a, b). These results suggest that the

bound 125 I-QC has been released from the juxtaglomerular cell together with that granule.

Although most of the observed reduction in QC release in the ischemic kidney probably results from the release of QC from the JG-cells, some other must be considered. First the ischemic kidney, due to the arterial ligation, probably contains less blood than the untouched kidney. However the volume of blood that could be present in a volume of kidney cortical tissue must be very small and contribute only a few cpm. The negligible amount compared to the total present in the untouched kidney. Second the ischemic kidney is not filtering. Therefore the preponderance of urine in the punctures might be the same in the two situations. However the kidney only excretes very small fractions of injected QC (1966 Thompson & Werbel 1972) and therefore the presence or absence of small amounts of QC in primary urine in the punctures probably contributes very little to the total amount of 125 I-QC. Therefore the major part of the difference between the ischemic and untouched kidney is probably due to release of granule content and 125 I-QC from the JG-cells.

In hemorrhagic shock experiments with a QC dose of 1 μ Ci and a shock period of 15 min or revealed no difference between control animals and experimental animals (Fig. 4). One possible explanation would be that no release has taken place. However since hemorrhage is known to increase release and since a sharp reduction of 125 I and 125 I-QC content is seen after ischemia, it also is known to induce renin-release, this explanation seems unlikely. Other factors might be due to consider. It has been described by several authors that the JG-cell is able to synthesize renin very rapidly (Thurau et al. 1972, Ver et al. 1975). Such very rapid renin-activation has been found to be induced by acute hemorrhage in total renal ischemia (Weber et al. 1975). Since renin is found exclusively inside the storage vesicles (Jaffar & Hartroft 1961) a rapid increase in renin also possibly imply that a rapid maturation of new granules has occurred. If sufficiently concentrated QC is present in the circulation, hemorrhage induced the rapid formation of new granules, the new granules would bind the QC. Therefore even if a release of granular QC content, sufficient to cause a reduced QC JGI reading, has taken place it would be hidden by a simulta-

neous increase of QC JGI due to formation of new QC-binding granules. This mechanism probably operates in the ischemic situation as well with one important difference: the artery ligation makes it impossible for circulating QC to reach the implicated newly formed granules which therefore could not be "stained" by QC. The reduction of QC fluorescent granules will thus not be hidden in the same way as in the shock situation. Although the amount of radioactivity measured in plasma after a single injection of 125 I-QC is not only produced by circulating labelled QC but also by possible labelled metabolites of the drug, the diagram in Fig. 6 demonstrates that it is quite possible that a sufficient amount of QC is still circulating 1-2 h after an 125 I-QC injection for QC binding to newly formed granules to occur. Moreover hemorrhagic shock causes activation of several endocrine cell systems. Therefore a possible release of QC from e.g. adrenal medullary cells and other sources can increase the circulating level of QC.

The above interpretation of the data is supported when using a longer QC-dose. Then QC JGI values in shocked animals were indeed found to be decreased ($p < 0.001$, Fig. 5). This decrease indicates that a release of QC has taken place and that newly formed granules have not been marked by QC. Thus with this technique the reduction of amounts of granules that were stained several hours before onset of bleeding is studied.

In conclusion, it is possible to show that acute renal ischemia, as well as acute hemorrhagic shock induces a decrease of QC JGI which most probably reflects a release of JG-cell granule content. Therefore it is possible that the recently reported *in vivo* QC binding to storage granules of certain endocrine cells (Ålund & Olsson 1976b) can also be used to study endocrine cells in the way it has been used in the present study as a marker of JG-cell activity.

Supported by the Swedish Medical Research Council (04X-81185), Sjöström Stiftelsen och Richard Jönhns fond, Lennander's fond, Karolinska Institute's funds and Magnus Bergvall's bequest.

I am very grateful to Mrs. Lena Hultgren and Mrs. Birgitta Ålund for the skilful technical assistance. I would also like to thank Dr. Olsson and Merid Hansberger. They have provided me with many valuable critical remarks. For secretarial help Mrs. Louise von P. von H. is gratefully acknowledged.

REFERENCES

- ALBERT A 1966 The acridines. Their preparation, physical, chemical and biological properties and uses. Edward Arnold Ltd. London.
- ÅLUND M 1978 Semiquantitative estimations of quinacrine fluorescence in intestinal nerve fibres. *Histochemistry* 58, 151-161.
- ÅLUND M & OLSON L 1978 Characterization of quinacrine-binding gastro-intestinal nerve fibres: a fluorescence histochemical method for non-adrenergic non-cholinergic nerves. In *Abstracts of II European Neurosci Meet*, Florence, September 4-9 1978. *Neuroscience Letters* Suppl 1 S265.
- ÅLUND M & OLSON L 1979a Depolarization-induced decreases in fluorescence intensity of gastro-intestinal quinacrine-binding nerves. *Brain Research* 166, 121-137.
- ÅLUND M & OLSON L 1979b Quinacrine affinity to endocrine cell systems containing dense core vesicles as visualized by fluorescence microscopy. *Cell and Tissue Research* 204, 171-186.
- ÅLUND M & OLSON L 1979c Quinacrine marker of nerves and endocrine cells with large dense core vesicles? In *Abstracts of III European Neurosci Meet*, Rome, September 11-14 1979. *Neuroscience Letters* Suppl III S130.
- ASSAYKEEN T A, CLAYTON P L, GOLDFIEN A & GANONG W F 1970 Effects of alpha and beta-receptor adrenergic blocking agents on the renin response to hypoglycemia and epinephrine in dogs. *Endocrinology* 87, 1318-1322.
- BLAINE E H, DAVIS J O & WITTY R T 1970 Renin release after hemorrhage and after suprarenal aortic constriction in dogs without sodium delivery to the macula densa. *Circ Res* 27, 1081-1089.
- BLAINE E H & DAVIS J O 1971 Evidence for a renal vascular mechanism in renin release: new observations with graded stimulation by aortic constriction. *Circ Res* 28-29 Suppl II 11118-11126.
- BLAINE E H, DAVIS J O & PREWITT L 1971 Evidence for a renal vascular receptor in control of renin secretion. *Am J Physiol* 220, 1593-1597.
- BURNSTOCK G, COOKS T & CROWE R 1978a Evidence for parasympathetic innervation of the avian ciliary muscle. *Brit J Pharmacol* 64, 13-20.
- BURNSTOCK G, COOKS T, CROWE R & KASAKOW L 1978b Parasympathetic innervation of the guinea-pig urinary bladder. *Brit J Pharmacol* 63, 125-138.
- BURNSTOCK G, CROWE R & WONG H 1979 Comparative pharmacological and histochemical evidence for parasympathetic inhibitory innervation of the portal vein of the rabbit but not guinea-pig. *Brit J Pharmacol* 68, 377-388.
- CORRODI H & JONSSON G 1967 The formaldehyde fluorescence method for the histochemical demonstration of biogenic monoamines. A review on the methodology. *J Histochem Cytochem* 15, 65-78.
- CORRODI H, FUXE K, LIDBRINK P & OLSON L 1971 Major tranquilizers: stress and central catecholamine neurons. *Brain Res* 29, 1-16.
- DAVIS J O & FREEMAN R M 1974 Regulation of renin release. *Physiological Reviews* 54, 51-74.
- EDELMAN W & HARTROFT P M 1964 Location of renin in juxtaglomerular cells of cat: through the use of the fluorescent antibody technique. *Circ Res* 9, 1069-1077.
- FALCK B, HILLARP N Å, THIESS G & ÅLUND M 1962 Fluorescence of catecholamines and compounds condensed with formaldehyde. *J Chem Cytochem* 10: 345-354.
- FARNEBO L O & HAMBERGER B 1971 Renal mechanisms in hemorrhage shock. *Acta Chir Scand* 143, 1-8.
- GOLDBLATT H, LYNCH J, HANZAL J, SUMMERVILLE W W 1934 Studies on renal hypertension. I. The production of persistent hypertension by means of renal artery constriction. *J Exp Med* 59, 347-379.
- HARTROFT P M & HARTROFT W S 1963 Renin release from juxtaglomerular cells. I. Variations with sodium chloride and desoxycorticosterone. *Experientia* 19, 415-427.
- JOHNSON J A, DAVIS J O & WITTY R T 1971 Effects of catecholamines and renal nerve section on renin release in the non-filtering kidney. *Circ Res* 29, 646-653.
- JOHNSON R G, SCARPA A & SALGANIKOFF M 1978 The internal pH of isolated secretory granules of pig platelets. *J Biol Chem* 253, 704-708.
- OLSON L & ÅLUND M 1979 Quinacrine fluorescence in the mouse aortic-coccygeal nerves: presence in the mouse aortic-coccygeal nerves after muscle transection. *J Biol Chem* 254, 182-186.
- OLSON L & UNGERSTEDT U 1970 A simple capacity freeze-drier for histochemical and chemical studies. *J Histochem Cytochem* 18, 8-19.
- OLSON L, ÅLUND M & NORBERG K 1978 Fluorescence microscopical demonstration of localization of gastrointestinal nerve fibres with an affinity for quinacrine. *Cell Tiss Res* 171, 407-410.
- REID I A, MORRIS B J & GANONG W F 1970 The renin-angiotensin system. *Ann Rev Physiol* 32, 377-410.
- SJÖSTRAND F 1944 Über die Eigenschaften tierischer Gewebe mit besonderer Berücksichtigung der Säugetiere. (Thesis) P. A. Norstedt Stockholm.
- THOMPSON P E & WERBEL L M 1977 Renal agents. Academic Press, New York and London.
- THURAU H W C, DAHLHEIM H, GRÜNER M, MASON J & GRANGER P 1972 Activation of renin in the single juxtaglomerular apparatus by sodium chloride in the tubular fluid at the macula densa. *Circ Res* 30-31 Suppl II 11187-11188.
- VANDER A J 1965 Effect of catecholamines and renal nerve on renin secretion in anesthetized dogs. *Am J Physiol* 209, 659-666.
- WEBER P, HELD E, UHLICH E & FIGLER J C 1975 Reaction constant of renin in the juxtaglomerular apparatus and plasma renin activity in renal ischemia and hemorrhage. *Kidney Int* 7, 331-341.

11. HUGHAM H & WEIL M H 1966. A model for the study of hemorrhagic shock in the rat. development of the method. *J Appl Physiol* 21 1860-1862.
12. SON W 1952. A new staining method for demonstrating the granules of the juxtaglomerular complex. *Anat Rec* 11 497-507.
13. WINER N, CHOKSHI D S., YOON M S. & FREEDMAN A. D 1969. Adrenergic receptor mediation of renin secretion. *J Clin Endocrinol Metabol* 29 1168-1175.

REFERENCES

- ALBERT A 1966 The acridines. Their preparation, physical, chemical and biological properties and uses. Edward Arnold Ltd, London.
- ÅLUND M 1978 Semiquantitative estimations of quinacrine fluorescence in intestinal nerve fibres. *Histochemistry* 58: 153-161.
- ÅLUND M & OLSON L 1978 Characterization of quinacrine-binding gastro-intestinal nerve fibres: a fluorescence histochemical method for non-adrenergic non-cholinergic nerves. In *Abstracts of II European Neurosci Meet*, Florence, September 4-9 1978. *Neuroscience Letters* Suppl 1: S265.
- ÅLUND M & OLSON L 1979a Depolarization induced decreases in fluorescence intensity of gastro-intestinal quinacrine binding nerves. *Brain Research* 166: 121-137.
- ÅLUND M & OLSON L 1979b Quinacrine affinity to endocrine cell systems containing dense core vesicles as visualized by fluorescence microscopy. *Cell and Tissue Research* 704: 171-186.
- ÅLUND M & OLSON L 1979c Quinacrine marker of nerves and endocrine cells with large dense core vesicles? In *Abstracts of III European Neurosci Meet*, Rome, September 11-14 1979. *Neuroscience Letters* Suppl 111: S130.
- ASSAYEEN T A, CLAYTON P L, GOLDSTEIN A & GANONG W F 1970 Effects of alpha and beta-receptor adrenergic blocking agents on the renin response to hypoglycemia and epinephrine in dogs. *Endocrinology* 87: 1318-1322.
- BLAINE F H, DAVIS J O & WITTY M T 1970 Renin release after hemorrhage and after suprarenal aortic constriction in dogs without sodium delivery to the macula densa. *Circ Res* 77: 1081-1089.
- BLAINE F H & DAVIS J O 1971 Evidence for a renal vascular mechanism in renin release: new observations with graded stimulation by aortic constriction. *Circ Res* 28-29 Suppl II: III18-III 6.
- BLAINE F H, DAVIS J O & PRIWITT L 1971 Evidence for a renal vascular receptor in control of renin secretion. *Am J Physiol* 220: 1393-1397.
- BURNSTOCK G, COOKS T & CROW R 1978a Evidence for purinergic innervation of the anococcygeus muscle. *Brit J Pharmacol* 64: 13-20.
- BURNSTOCK G, COOKS T, CROW R & KASAKOW L 1978b Puriergic innervation of the guinea-pig urinary bladder. *Brit J Pharmacol* 63: 125-138.
- BURNSTOCK G, CROW R & WONG H 1979 Comparative pharmacological and histochemical evidence for purinergic inhibitory innervation of the portal vein of the rabbit but not guinea-pig. *Brit J Pharmacol* 65: 377-388.
- CORRODI H & JONSSON H 1967 The formaldehyde fluorescence method for the histochemical demonstration of biogenic monoamines. A review on the methodology. *J Histochem Cytochem* 15: 65-78.
- CORRODI H, FUXE K, LIDBRINK P & OLSON L 1971 Minor tranquillizers: stress and central catecholamine neurons. *Brain Res* 79: 1-16.
- DAVIS J O & TRIEMAN R M 1976 Medullary regulating renin release. *Physiological Reviews* 56: 1-31.
- EDLIMAN R & HARTROIT P M 1961 Isolation of renin in juxtaglomerular cells of rabbit kidney through the use of the fluorescent antibody technique. *Circ Res* 9: 1069-1077.
- JALCK B, HILLARP N Å, THILMÉ, O & ÅLUND A 1962 Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J Histochem Cytochem* 10: 348-354.
- GARNIERO L O & HAMBERGER B 1978 A reninergic mechanism in hemorrhagic shock of the rat. *Acta Chir Scand* 143: 1-8.
- GOLDMATT H, LYNCH J, HANZAL R & SUMMERVILLE W W 1934 Studies on experimental hypertension. I. The production of persistent elevation of systolic blood pressure by means of renal chemia. *J Exp Med* 59: 347-379.
- HARTROIT P M & HARTROIT W S 1953 Sodium on juxtaglomerular cells. I. Variations produced by sodium chloride and desoxycorticosterone acetate. *Experientia* 9: 415-417.
- JOHNSON J A, DAVIS J O & WITTY M T 1971 Effects of catecholamines and renal nerve stimulation on renin release in the non-filtering kidney. *Circ Res* 29: 646-653.
- JOHNSON R G, SCARPA A & SALGANCHIKOV 1978 The internal pH of isolated serotonergic granules of pig platelets. *J Biol Chem* 253: 7061-7065.
- OLSON L & ÅLUND M 1979 Quinacrine in nerves: presence in the mouse non-coccygeus and disappearance after muscle transection. *Neurobiology* 57: 182-186.
- OLSON L & UNGERSTEDT U 1970 A simple capacity freeze-drier for histochemical use. *Physiology* 2: 8-19.
- OLSON L, ÅLUND M & NORBERG A 1978 Fluorescence microscopical demonstration of isolation of gastrointestinal nerve fibres with a selective affinity for quinacrine. *Cell Tiss Res* 171: 407-425.
- RIGD I A, MORRIS B J & GANONG W F 1971 The renin-angiotensin system. *Ann Rev Physiol* 40: 377-410.
- SJÖSTRAND F 1944 Über die Eigenschaften tierischer Gewebe mit besonderer Berücksichtigung der Säugelkerniere (Thesis). P. A. Norstedt och Stockholm.
- THOMPSON P I & WERBEL I M 1972 *Arterial agents*. Academic Press, New York and London.
- THURAU K W C, DAHLGREN H & GRUNER MASON J & GRANOFF P 1972 Activation of renin in the single juxtaglomerular apparatus: sodium chloride in the tubular fluid at the macula. *Circ Res* 30-31 Suppl II: II 18-II 166.
- VANDER A J 1963 Effect of catecholamines and renal nerve on renin secretion in anesthetized dog. *Am J Physiol* 209: 619-666.
- WEBER P, HILD I, UHLICH I & FIEGLER C 1975 Reaction constant of renin in the juxtaglomerular apparatus and plasma renin activity: renal ischemia and hemorrhage. *Kidney Internat* 7: 331-341.

the effect of dibutyl cAMP and PGE₂ on lysosomal enzyme release and lactate production in relation to bone resorption in vitro

LERNER

Department of Oral Pathology, University of Umeå, Sweden

LERNER, U. The effect of dibutyl cyclic AMP and PGE₂ on lysosomal enzyme release and lactate production in relation to bone resorption in vitro. *Acta Physiol Scand* 1980 110: 123-130. Received 27 Dec 1979. ISSN 0001-6772. Department of Oral Pathology, University of Umeå, Sweden.

The effect of dibutyl cyclic AMP (dbcAMP) and PGE₂ on the content and release of lysosomal and non-lysosomal enzymes was studied in a bone organ culture system using half calvaria from 6-7-day-old mice. I parallel the effect of dbcAMP and PGE₂ on the release of calcium (Ca²⁺) and inorganic phosphate (P), glucose consumption and lactate production was also followed. DbcAMP (2.5 · 10⁻⁴ M) decreased the release of β -glucuronidase, β -N-acetyl-glucosaminidase, acid phosphatase, Ca²⁺ and P during the first day of culture. During the 3rd and 4th day dbcAMP increased all these parameters. In contrast no changes in the release of lactate dehydrogenase (LDH) and alkaline amino-transferase (ALAT) were seen. Glucose consumption and lactate production was not stimulated by dbcAMP until the 3rd and 4th day. On the other hand, PGE₂ (10⁻⁶ M) stimulated the release of β -glucuronidase, β -N-acetylglucosaminidase, Ca²⁺ and P as well as glucose consumption and lactate production already after 4 h and this stimulation was maintained throughout the culture period. No effect by PGE₂ on the release of LDH and ALAT was registered. The activities of LDH in the bone explants after 96 h of culture were significantly increased by both dbcAMP and PGE₂. It is concluded that bone resorption stimulated by dbcAMP and PGE₂ is associated with increased lysosomal enzyme release and lactate production.

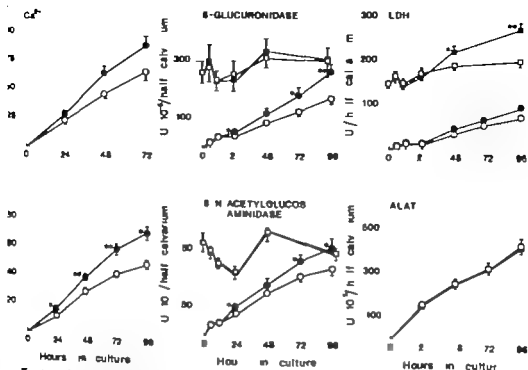
Key words: Bone resorption, lysosomal enzyme release, lactate, PGE₂, cyclic AMP

cAMP (cAMP) is considered to be an intracellular mediator of the bone resorptive effect of parathyroid hormone (PTH) and prostaglandins of E-type (PGE₁, PGE₂) (see review by Rasmussen & Border 1974). We have studied the effect of cAMP on bone resorption in vitro and our results indicate that cAMP, in contrast to PTH, PGE₁ and PGE₂, stimulates bone resorption only after an initial lag period or a period of reduced bone resorption (Lerner & Gustafson, to be published). Thus we consider cAMP to be a possible mediator only of a delayed effect of PTH, PGE₁ and PGE₂, probably related to the capacity of PTH and PGE₂ to recruit new osteoclasts. The cAMP induced inhibition of spontaneous mobilization of calcium and inorganic phosphate during the initial stages was found to be associated with a parallel reduction in lysosomal enzyme activities recovered from the culture media (Lerner & Gustafson 1979; Lerner

1979a). When PGE₂-stimulated bone resorption was inhibited by cAMP, a parallel reduction of mineral mobilization and lysosomal enzyme release was also seen during the first 48 h (Lerner & Gustafson 1979b).

Our finding that cAMP has the capacity to inhibit lysosomal enzyme release agrees with the well-known inhibitory effect of cAMP on release processes of many other cells, e.g. mast cells (Lichtenstein 1974; Orange et al. 1971), neutrophils (Ignarro 1973; Zinner et al. 1974) and macrophages (Weissman et al. 1971) but differs from two studies which have been done on bone (Vaes 1968a; Eldon & Raisz 1978).

In the experiments described in the present report we have extended the culture period to 96 h and have examined the effect of dibutyl cyclic AMP (dbcAMP) and PGE₂ upon both mineral and lysosomal enzyme release from bone calvaria. As



4. Effects of the effect of 10^{-6} M PGE_2 on the release of calcium and inorganic phosphate activities of β -glucuronidase, β -N-acetylglucosaminidase, LDH and ALAT in media and bone explants from cultures of calvarial groups of half calvaria (6-8 paired bones) were harvested after 0, 6, 12, 24, 48 and 96 h and the enzyme activities assayed in media (circles) and bones (squares). In addition mineral analyses were performed in the media. The rates of ALAT in the bones were only determined after 96 h. The control bones contained 105 ± 14 U 10^{-4} /half calvarium and the bones cultured in the presence of PGE_2 99 ± 7 U 10^{-4} /half calvarium. Open symbols represent values from control cultures and filled symbols values from cultures exposed to PGE_2 . Values are mean and SEM is given as a vertical bar. $P < 0.05$ $^{**}P < 0.01$.

not in itself influence the release of ^{45}Ca from the

RESULTS

Effect of dbcAMP and PGE_2 on the release of ^{45}Ca and P

The calvarial bones were cultured in the presence of 2.5×10^{-6} M dbcAMP. A strong, significant release of the release of Ca^{2+} and P was seen during the first 4 h. During the second day of culture no changes were registered but during the subsequent two days significantly increased release of both Ca^{2+} and P was obtained (Fig. 1). Addition of PGE_2 to culture medium (10^{-6} M) resulted in stimulation of the release of Ca^{2+} after 48 h and P already after 4 h and this stimulation was maintained during the complete culture period (Fig. 1). In other experiments, not demonstrated in this paper, a significant release of both Ca^{2+} and P has

been obtained after 24 h when PGE_2 (10^{-6} M) was present in culture medium.

Effect of dbcAMP and PGE_2 on the release of lysosomal enzyme

The activity of β -glucuronidase in media from bones cultured in the presence of dbcAMP (5×10^{-6} M) was decreased during the initial 24 h but significantly enhanced during the following three days (Table 1). The bone explants exposed to dbcAMP (2.5×10^{-6} M) for 96 h did not show any differences in the activity of β -glucuronidase as compared with untreated paired controls (Table 1). The activity in the total culture (bone after culture + medium) was significantly increased when the bones had been cultured in the presence of dbcAMP (Table 1).

When the activities of acid phosphatase and β -N-acetylglucosaminidase were assayed in the cal-

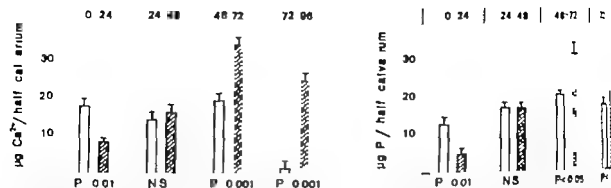


Fig. 1. Kinetics of the effect of dbcAMP (2.5×10^{-4} M) on the spontaneous release of calcium (left) and inorganic phosphate (right) from calvarial bones cultured for 96 hours. Open columns represent values from control cultures, filled columns from cultures with dbcAMP. Values are means \pm S.E. for 7 paired expts.

Increased lactate production has been shown to be associated with PTH stimulated bone resorption (Vaes 1968b); glucose consumption and lactate production were also registered.

MATERIALS AND METHODS

Tissue culture procedure

Calvarial (frontal and parietal) bones from 6–7-day-old mice (C57 type) were dissected aseptically and washed in Tyrode's solution. Histological examination of sections of fixed and stained calvaria showed that they were free from blood and vascular cells and that the bones had a thin layer of periosteal cells covering the outer and inner surfaces. Care was taken when the dissection procedure not to damage the thin periosteum layer. The calvaria were divided along the sagittal suture giving two halves, one of which was used as a control bone and the other as an experimental bone. The paired calvarial halves were then placed on separate stainless steel grids (Falcon 3014, Falcon Plastics, Los Angeles, USA) in multiwell culture dishes (Linbro Scientific Inc., Hamden, Conn., USA) with 1 ml of a modification of the chemically defined medium CMRL 1066 containing 11% albumin (w/v). Then dishes were put into plexiglass chambers and gassed with 5% CO₂ in air. The stationary cultures were maintained at 37°C for 96 h. The media were changed every 4 h. After culture the calvarial bones were placed in 0.2% (w/v) Triton X100 in saline for 1 h at 4°C and the supernatant used for enzyme assays. For further details, see Lerner & Gustafson 1979a.

Quantification of bone resorption

The magnitude of bone resorption was assessed by following the increase in concentration in the media of calcium (Ca) and inorganic phosphate (P). Ca was analysed according to Wilks (1970) and P according to Chen et al. (1956).

Enzyme assays

The release of lysosomal enzymes from the bones was followed by registering the activities of β -glucuronidase, β -N-acetylglucosaminidase and acid phosphatase in the

media and the bones after culture. The release of lysosomal enzymes was studied by determining activities of lactate dehydrogenase (LDH) and α -amino transferase (ALAT) in the same samples as in.

β -glucuronidase (EC 3.2.1.31) was determined phenolphthalein-glucuronide as substrate (Vaes & Jacques 1965). β -N-acetylglucosaminidase (EC 3.2.1.9) was assayed with p-nitrophenyl N-acetyl- β -D-glucosaminide as substrate (Vaes & Jacques 1965) and the liberated p-nitrophenol measured according to Sellinger et al. (1962). Acid phosphatase (EC 3.1.3.2) was determined by amount of p-nitrophenol liberated from p-nitrophenyl phosphate (Barrett 1977). LDH (EC 1.1.1.27) was assayed by determining the rate of oxidation of nicotinamide adenine dinucleotide at 340 nm and (Wroblewski & LaDue 1955). ALAT (EC 2.6.1.2) was assayed at 37°C according to Kabi Diagnostica (1974). Assays were performed under conditions where the reaction was linear with respect to time and enzyme concentration. Unit refers to the decomposition 1 μ mol substrate⁻¹ min⁻¹.

Other assays

Glucose consumption was estimated by determining decrease of glucose consumption in the media. Glucose was determined by the coupled hexokinase-glucose-6-phosphate dehydrogenase method (Barthelme & C. 1962). Lactate in the media was analysed according to Marbach & Weil (1967).

Material

Essentially fatty acid free bovine serum albumin (Boehringer-Mann), Triton X100, phenolphthalein, glucuronide, p-nitrophenyl N-acetyl- β -D-glucosaminide, p-nitrophenyl phosphate, pyruvate, NADH, hexokinase, yeast glucose-6-phosphate dehydrogenase, (yeast) NADP, α -dehydrogenase (beef heart), NAD and N⁶-carboxyadenosine 3',5'-cyclic monophosphate were from Sigma Chemical Co., St. Louis, Mo., USA. Alanine, α -oxo-glutarate and pyridoxal phosphate were from Kabi, Stockholm, Sweden. PGE₁ (kindly supplied by the Upjohn Co., Kalamazoo, MI) through the courtesy of Dr. John F. Pike) was dissolved in ethanol. The ethanol concentration in the experimental and control culture medium never exceeded 0.1%, a concentration

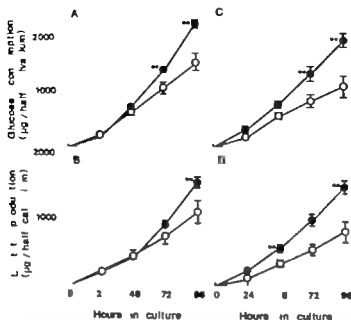


Fig. 3 Kinetics of the effect of 5×10^{-6} M dbcAMP (A, B) and 10^{-6} M PGE_2 (C, D) on glucose consumption and lactate production in 96-hour cultures of calvarial bones. Open circles represent bones from control cultures and filled circles are from cultures treated with dbcAMP or PGE_2 . Values are means from 9 paired experiments and SEM is given as vertical bar. Bars larger than the symbol height, $^*P < 0.05$, $^{**}P < 0.01$.

Effect of dbcAMP and PGE_2 on the release of lysosomal enzymes

Table 2 it appears that the presence of dP in the culture media (2.5×10^{-6} M) did not cause any changes in the release of LDH and ALP. However, both in the bones after 96 h of culture and in the total culture, an augmentation of amounts of LDH was registered while no differences in the activities of ALAT were obtained.

10^{-6} M PGE_2 did not produce any changes in the release of LDH and ALAT from the bones to the culture media (besides a small significant increase in LDH after 96 h). However, in the bones, a stimulation of the activity of LDH was seen after 48 h and in contrast, no changes of ALAT was registered in the explants (Fig. 3).

Effect of dbcAMP and PGE_2 on glucose consumption and lactate production

As seen in Fig. 3 both glucose consumption and lactate production was unaltered during the first 24 h when the bones were cultured in the presence of dbcAMP (2.5×10^{-6} M). During the subsequent 4 days bones exposed to dbcAMP con-

sumed more glucose and produced more lactate than untreated bones.

Addition of PGE_2 to culture media (10^{-6} M) resulted in increased glucose consumption and lactate production during all the four days (Fig. 3).

DISCUSSION

Lysosomal enzymes and bone resorption

Our finding that bone resorption promoted by PGE_2 is associated with an increased release of lysosomal enzymes is in agreement with recent observations by Eilon & Raisz (1978). The additional finding that dbcAMP enhanced the release of three lysosomal enzymes in parallel with stimulation of bone resorption confirms the results reported by Vacek (1968a). Eilon & Raisz (1978) and Lerner & Gustafson (1979c). However, in contrast to PGE_2 , dbcAMP caused an initial reduction of lysosomal enzyme release and mineral mobilization, an observation which agrees with earlier results from this laboratory (Lerner & Gustafson 1979). The effects of PGE_2 and dbcAMP on enzymes from lysosomal stores are specific, since the release of the non-

Table 1 The effect of dbcAMP (5×10^{-6} M) on the content and release of lysosomal enzymes from calvarial bones maintained in organ culture for 96 hValues are means \pm SE for 7-9 paired experiments

	Medium				Bone after 96 h	Total culture
	0- 4 h	4-48 h	48-72 h	72-96 h		
β-glucuronidase						
(U $\times 10^{-6}$ /half calvarium)						
Control	3.9 \pm 0.8	25.7 \pm 3.0	74.4 \pm 9	17.6 \pm 0	161 \pm 16	341
dbcAMP	2.2 \pm 0.3	49.3 \pm 5.7	51.0 \pm 4.8*	42.0 \pm 6.4	195 \pm 32	348
Acid phosphatase						
(U $\times 10^{-7}$ /half calvarium)						
Control	29.0 \pm 5.0	18.0 \pm 2.6	19.5 \pm 1.6	15.8 \pm 2.7	1617 \pm 29	1699
dbcAMP	14.4 \pm 4.3	26.4 \pm 5	49.5 \pm 7.4	35.4 \pm 3.4	2576 \pm 264	760
β-N-acetylglucosaminidase						
(U $\times 10^{-4}$ /half calvarium)						
Control	61.8 \pm 4.3	31.7 \pm 2.4	44.3 \pm 3.1	37.1 \pm 3.4	190 \pm 18	365
dbcAMP	71.8 \pm 0.7	76.6 \pm 1.3	57.2 \pm 3.6	40.2 \pm 6.6	14 \pm 16	368

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$
 Bone after culture + media

ture media the kinetic pattern was similar to that seen when β glucuronidase was registered. Thus the presence of dbcAMP (5×10^{-6} M) in the culture media resulted in a strong inhibition during the first 24 h. During the second day no striking differences were discernible but during the third and fourth day the activities in the media from bones exposed to dbcAMP were enhanced although the effect as regards to the activities of β N-acetylglucosaminidase was not too pronounced (Table 1). The amounts of acid phosphatase in the bones after 96 h of culture were significantly increased after addition of dbcAMP to the culture media as were the activities of acid phosphatase in the total culture (Table 1).

When the bone explants were treated with PGE_2 a stimulation of the release of β glucuronidase and β N-acetylglucosaminidase registered after 24 h and this stimulation was maintained for 96 h (Fig. 7). No differences in activities of β glucuronidase and β N-acetylglucosaminidase between PGE_2 -treated and control bones were registered during the culture period (Fig. 7). It can also be seen from Fig. 7 that control and experimental bones released similar amounts of the two acid hydrolases. Despite this there were no changes in the activities in the media after 96 h as compared with bones from both as regards control and experimental bones.

Table 2 The effect of dbcAMP (2.5×10^{-6} M) on the content and release of LDH and ALAT from bones maintained in organ culture for 96 hValues are means \pm S.E. for 9 paired experiments

	Medium				Bone after culture	Total culture
	0-4 h	4-48 h	48-72 h	72-96 h		
LDH (U/half calvarium)						
Control	79.6±1.9	1.7±0.6	25.3±1	4.4±0.8	789±12	999±13
dbcAMP	3.9±0.4	30.4±2.2	79.3±8	18.2±7	377±70*	794±17
ALAT (U×10 ⁻⁷ /half calvarium)						
Control	134±9	143±11	116±5	109±13	163±15	665±11
dbcAMP	137±6	147±1	104±11	87±1	187±13	661±1

* $P < 0.001$
 Bone after culture + media.

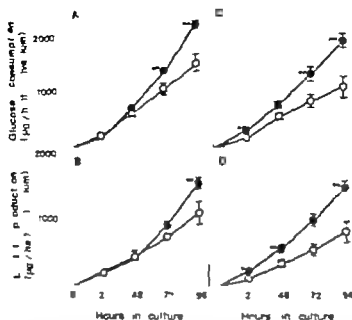


Fig. 3. Kinetics of the effect of 10^{-6} M dbcAMP (A, B) and 10^{-6} M PGE_2 (C, D) on glucose consumption and lactate production in 96-hour cultures of calvarial bones. Open circles represent bones from control cultures and filled circles are from cultures treated with dbcAMP or PGE_2 . Values are means from 9 paired experiments and SEM given as a vertical bar, here larger than the symbol height. $P < 0.05$ vs. $P < 0.01$.

Effect of dbcAMP and PGE_2 on the release of lysosomal enzymes

Table 1. It appears that the presence of cAMP in the culture media (2.5×10^{-6} M) did not cause any changes in the release of LDH and ALAT. However, both in the bones after 96 h of culture and in the total culture, an augmentation of activity of LDH was registered, while no differences in the activities of ALAT were obtained. PGE_2 did not produce any changes in the release of LDH and ALAT from the bones to the culture media (besides a small significant increase after 96 h). However, in the bones, stimulation of the activity of LDH was seen after 48 h and, in contrast, no changes of ALAT was registered in the explants (Fig. 1).

Effect of dbcAMP and PGE_2 on glucose consumption and lactate production

As can be seen in Fig. 3, both glucose consumption and lactate production was unaltered during the first two days when the bones were cultured in the presence of dbcAMP (2.5×10^{-6} M). During the subsequent two days, bones exposed to dbcAMP con-

sumed more glucose and produced more lactate than untreated bones.

Addition of PGE_2 to culture media (10^{-6} M) resulted in increased glucose consumption and lactate production during all the four days (Fig. 3).

DISCUSSION

Lysosomal enzymes and bone resorption

Our finding that bone resorption promoted by PGE_2 is associated with an increased release of lysosomal enzymes is in agreement with recent observations by Eilon & Razin (1979). The additional finding that dbcAMP enhanced the release of three lysosomal enzymes in parallel with stimulation of bone resorption confirms the results reported by Vais (1968a). Eilon & Razin (1978) and Lerner & Gustafson (1979a). However, in contrast to PGE_2 , dbcAMP caused an mutual reduction of lysosomal enzyme release and mineral mobilization, an observation which agrees with earlier results from this laboratory (Lerner & Gustafson 1979a). The effect of PGE_2 and dbcAMP on enzymes from lysosomal stores are specific, since the release of the non-

Table 1 The effect of dbcAMP (2.5×10^{-4} M) on the content and release of lysosomal enzymes from calvarial bones maintained in organ culture for 96 hValues are means \pm SE for 7-9 paired expts

	Medium				Bone after 96 h	Total culture
	0-24 h	4-48 h	48-72 h	72-96 h		
β -glucuronidase ($U \times 10^{-4}$ /half calvarium)						
Control	3.9 \pm 0.8	25.7 \pm 3.0	4.4 \pm 2.9	17.6 \pm 0	161 \pm 16	34
dbcAMP	2.2 \pm 0.3	49.3 \pm 5.7	51.0 \pm 4.8	4.0 \pm 6.4	194 \pm 32	349 \pm 3
Acid phosphatase ($U \times 10^{-4}$ /half calvarium)						
Control	79.0 \pm 5.0	18.0 \pm 6	19.5 \pm 1.6	15.8 \pm 1.7	1617 \pm 229	1697
dbcAMP	14.4 \pm 4.3	26.4 \pm 5	49.5 \pm 7.4	35.4 \pm 3.4	7576 \pm 284	7670
β -N acetylglucosaminidase ($U \times 10^{-4}$ /half calvarium)						
Control	61.8 \pm 4.3	31.7 \pm 2.4	44.3 \pm 3.1	37.1 \pm 3.4	190 \pm 18	161
dbcAMP	71.8 \pm 0.7*	76.6 \pm 1.3	57.2 \pm 3.6	40.4 \pm 6.6	714 \pm 16	766

* $P < 0.05$ $P < 0.01$ $P < 0.001$

Bone after culture + media

ture media the kinetic pattern was similar to that seen when β glucuronidase was registered. Thus the presence of dbcAMP (2.5×10^{-4} M) in the culture media resulted in a strong inhibition during the first 24 h. During the second day no striking differences were discernible but during the third and fourth day the activities in the media from bones exposed to dbcAMP were enhanced although the effect as regards to the activities of β -N acetylglucosaminidase was not too pronounced (Table 1). The amounts of acid phosphatase in the bones after 96 h of culture were significantly increased after addition of dbcAMP to the culture media as were the activities of acid phosphatase in the total culture (Table 1).

When the bone explants were treated with PGE_2 a stimulation of the release of β glucuronidase and β -N-acetylglucosaminidase registered after 24 h and this stimulation was maintained for 96 h (Fig. 1). No differences in the activities of β -glucuronidase and β -N-acetylglucosaminidase between PGE_2 -treated and control bones were registered during the culture period (Fig. 2). It can also be seen from Fig. 1 that control and experimental bones released similar amounts of the two acid hydrolases. Despite this there were no changes in the activities in the media after 96 h as compared with bones from day 0 both as regards control and experimental bone.

Table 2 The effect of dbcAMP (2.5×10^{-4} M) on the content and release of LDH and ALAT from calvarial bones maintained in organ culture for 96 hValues are means \pm S.E. for 9 paired expts

	Medium				Bone after culture	Total culture
	0-24 h	24-48 h	48-72 h	72-96 h		
LDH (U/half calvarium)						
Control	29.6±1.9	3.7±.6	25.3±2.1	4.4±.8	289±1	399±11
dbcAMP	31.9±.4	30.4±2	29.3±.8	28.4±2.7	372±70*	494±17
ALAT (U×10 ⁻³ /half calvarium)						
Control	134±9	143±11	116±5	109±13	163±15	663±13
dbcAMP	137±6	147±12	104±11	87±12	187±13	663±1

* $P < 0.001$

Bone after culture + media

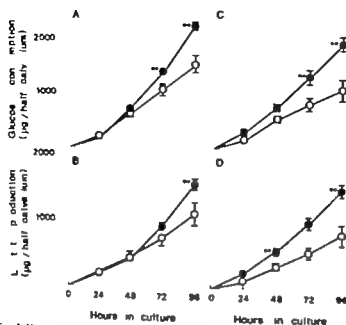


Fig. 3. Kinetics of the effect of $3 \cdot 10^{-4}$ M dbcAMP (A, B) and 10^{-6} M PGE_2 (C, D) on glucose consumption and lactate production in 96-hour cultures of calvarial bones. Open circles represent bones from control cultures and filled circles are from cultures treated with dbcAMP or PGE_2 . Values are means from 9 paired experiments and SEM given as vertical bar less than the symbol height. $*P < 0.05$, $**P < 0.01$.

Effect of dbcAMP and PGE_2 on the release of lysosomal enzymes

Table 2 it appears that the presence of $1P$ in the culture media ($2.5 \cdot 10^{-4}$ M) did not cause any changes in the release of LDH and ALAT. However, both in the bones after 96 h and in the total culture, an augmentation of amounts of LDH was registered, while no differences in the activities of ALAT were obtained. $1P$ PGE_2 did not produce any changes in the release of LDH and ALAT from the bones to the culture media (besides a small significant increase in LDH after 96 h). However, in the bones, a stimulation of the activity of LDH was seen after 48 h and 96 h. In contrast, no changes of ALAT were registered in the explants (Fig. 2).

Effect of dbcAMP and PGE_2 on glucose consumption and lactate production

As seen in Fig. 3 both glucose consumption and lactate production was enhanced during the 96 hours when the bones were cultured in the presence of dbcAMP ($2.5 \cdot 10^{-4}$ M). During the subsequent two days bones exposed to dbcAMP con-

sumed more glucose and produced more lactate than untreated bones.

Addition of PGE_2 to culture media (10^{-6} M) resulted in increased glucose consumption and lactate production during all the four days (Fig. 3).

DISCUSSION

Lysosomal enzymes and bone resorption

Our finding that bone resorption promoted by PGE_2 is associated with an increased release of lysosomal enzymes is in agreement with recent observations by Eilon & Ransz (1978). The additional finding that dbcAMP enhanced the release of three lysosomal enzymes in parallel with stimulation of bone resorption confirms the results reported by Vnes (1968a). Eilon & Ransz (1978) and Lerner & Gustafson (1979). However, in contrast to PGE_2 , dbcAMP caused an initial reduction of lysosomal enzyme release and mineral mobilization, an observation which agrees with earlier results from this laboratory (Lerner & Gustafson 1979). The effects of PGE_2 and dbcAMP on enzymes from lysosomal stores are specific, since the release of the non-

Table 1 The effect of dbcAMP (2.5×10^{-4} M) on the content and release of lysosomal enzymes in calvarial bones maintained in organ culture for 96 hValues are means \pm SE for 7-9 paired expts

	Medium				Bone after 96 h	Total culture
	0-24 h	4-48 h	48-72 h	72-96 h		
β-glucuronidase						
(U $\times 10^{-3}$ /half calvarium)						
Control	3.9 \pm 2.8	45.7 \pm 3.0	74.4 \pm 2.9	17.6 \pm 1.0	161 \pm 16	241
dbcAMP	22.2 \pm .3	49.3 \pm 5.7	51.0 \pm 4.8	42.0 \pm 6.4	195 \pm 3	39
Acid phosphatase						
(U $\times 10^{-3}$ /half calvarium)						
Control	29.0 \pm 5.0	18.0 \pm 7.6	19.5 \pm 1.6	15.8 \pm .7	1617 \pm 229	148
dbcAMP	14.4 \pm 4.3	76.4 \pm 2.5	49.5 \pm 7.4	35.4 \pm 3.4	576 \pm 241	70
β-N-acetylglucosaminidase						
(U $\times 10^{-3}$ /half calvarium)						
Control	61.8 \pm 4.3	31.7 \pm .4	44.3 \pm 3.1	37.1 \pm 3.4	190 \pm 18	51
dbcAMP	71.8 \pm 0.7*	76.6 \pm 1.3	57.4 \pm 3.6	40. \pm 6.6	14 \pm 16	54

* $P < 0.05$ $P < 0.01$ $P < 0.001$

Bone after culture + media

ture media the kinetic pattern was similar to that seen when β glucuronidase was registered. Thus the presence of dbcAMP (2.5×10^{-4} M) in the culture media resulted in a strong inhibition during the first 74 h. During the second day no striking differences were discernible but during the third and fourth day the activities in the media from bones exposed to dbcAMP were enhanced although the effect as regards to the activities of β N acetylglucosaminidase was not too pronounced (Table 1). The amounts of acid phosphatase in the bones after 96 h of culture were significantly increased after addition of dbcAMP to the culture media as were the activities of acid phosphatase in the total culture (Table 1).

When the bone explants were treated with PGE a stimulation of the release of β glucuronidase and β N acetylglucosaminidase was registered after 24 h and this stimulation was maintained for 96 h (Fig. 2). No differences in activities of β glucuronidase and β N acetylglucosaminidase between PGE₂ treated and control bones were registered during the culture (Fig. 2). It can also be seen from Fig. 2 that control and experimental bones released amounts of the two acid hydrolases. Despite this there were no changes in the activities in the media after 96 h as compared with bones from day 0 both as regards control and experimental bone.

Table 2 The effect of dbcAMP (2.5×10^{-4} M) on the content and release of LDH and ALAT from calvarial bones maintained in organ culture for 96 hValues are mean \pm S.E. for 9 paired expts

	Medium				None after culture	Total culture
	0-24 h	4-48 h	48-72 h	72-96 h		
LDH (U/half calvarium)						
Control	79.6 ± 1.9	32.7 ± .6	45.3 ± 2.1	24.4 ± 2.8	289 ± 1	199
dbcAMP	3.9 ± .4	30.4 ± .2	29.3 ± 2.8	28. ± 2.7	372 ± 70	494
ALAT (U × 10 ⁻³ /half calvarium)						
Control	134 ± 9	143 ± 11	116 ± 5	109 ± 13	163 ± 15	665
dbcAMP	137 ± 6	147 ± 1	104 ± 11	87 ± 1	187 ± 13	663

* $P < 0.001$

Bone after culture + media

local cells. Alternatively the augmentation of could be a consequence of an increased of cells. It should, however be pointed out the effect of dbcAMP and PGE₂ seems to be re to that degree that the activities of ALAT unchanged

work was supported by grant from the Swedish against Rheumatic Diseases, from the Royal Fund of King Oskar V and from the Swedish Research Council (project No. B79-14X-02426-

author gratefully acknowledges: M. Anita Johansson for Karlsson and Mrs Britta Lindgren for technical assistance. Ms Bergitta Wiklund and Mrs A. W. W. for typing the manuscript. Mrs Y. Öster for drawing the illustrations and Dr Ian Jones for his critical comments of the manuscript

REFERENCES

- ET AL. J. 1972 Lysosomal enzymes. In: Lysosomes. Laboratory handbook (ed J. T. Dingle), pp. 126. North-Holland Publishing Company, Amsterdam, London.
- HELMAL W. & CZOK, R. 1964. Enzymatische Veränderungen der Glucose in Blut, Liquor und Harn. *Wochenschr.* 40, 583-589.
- IMAGE, R. & NEUMAN W. F. 1979 Mechanisms mobilization of bone mineral by 1,25-dihydroxyvitamin D₃. *Am J Physiol* 237, E113-E120.
- J. P. S. TORIBARA, T. Y. & WARNER, H. 1956 Determination of phosphorus. *Anal Chem* 28, 1756-1758.
- MAN, R. A., RAMP, W. K., TOVERUD, S. U. & UKER, J. S. 1976 Electron microscopic localization of lactate dehydrogenase in osteoclasts of chick embryos. *Histochemistry* 51, 543-552.
- LE, J. T. 1978 Articular damage in arthritis and its relief. *Ann Intern Med* 88, 421-426.
- LE, J. T. 1979 Recent studies on the control of articular damage: the contribution of the Straubways research Laboratory. *Ann Rheum Dis* 38, 201-214.
- S. M. & SCHOFFELD, B. H. 1972 Electron microscopic localization of hydrolytic enzymes in osteoclasts. *Histochemistry* 34, 243-258.
- VAES, G. & RAISZ, L. O. 1978 Comparison of the effects of stimulators and inhibitors of resorption on release of lysosomal enzymes and radioactive calcium from fetal bone in organ culture. *Endocrinology* 102, 1968-1975.
- WILKINSON, E. D. J. & CARTWRIGHT, E. C. 1977 Connective tissue collagenases. In: *Proteins in animal cells and tissues* (ed A. J. Barrett), pp. 249-283. North-Holland Publishing Company, Amsterdam, New York, Oxford.
- WILKINSON, E. D. J. 1973 Neutral protease release from tissue leukocytes regulated by neurohormones and cyclic nucleotides. *Nature* 245, 131-133.
- WILKINSON, E. D. J. 1973 Manual for determinations of aspartate aminotransferase and alanine aminotransferase. *Kabi Diagnostics* Stockholm 1973.
- LERNER, U. 1979 Inhibition of bone resorption and lysosomal enzyme release from calvarial bones cultured for 4 hours: Synergism between cyclic AMP analogues and phosphodiesterase inhibitors. *Acta Endocrinol (Copenh)*, 1, preprint.
- LERNER, U. 1979b Relationship between bone resorption and lysosomal enzyme release as demonstrated by the effect of 1α(OH)D₃ in an organ culture system. *Biochem Biophys Acta*, 1, preprint.
- LERNER, U. & GUSTAFSSON, G. T. 1979a Laboratory effect of dibutyryl cyclic AMP on the release of calcium, inorganic phosphate and lysosomal enzymes from calvarial bones cultured for 4 hours. *Acta Endocrinol (Copenh)* 191, 710-74.
- LERNER, U. & GUSTAFSSON, G. T. 1979b A revised role for cyclic AMP in prostaglandin induced bone resorption. *Med Biol*, in press.
- LERNER, U. & GUSTAFSSON, G. T. 1979 Cyclic nucleotides and bone resorption. In: *New aspects of rheumatoid inflammation, an international workshop* (ed O. Lögren), pp. 35-36. *Scand J Rheumatol (Suppl)*.
- LICHTENSTEIN, L. M. 1974 The role of the cyclic AMP system in inflammation, an introduction. I. Cyclic AMP, cell growth and the immune response (ed W. Braun, L. M. Lichtenstein and C. W. Parker), pp. 147-16. Springer Verlag, New York.
- LUCHT, U. 1971 Acid phosphatase of osteoclast demonstrated by electron microscopic histochemistry. *Histochemistry* 28, 103-117.
- MARBACH, E. P. & WEILL, H. H. 1967 Rapid enzymatic measurement of blood lactate and pyruvate. *Clin Chem* 13, 314-323.
- ORANGE, R. F., AUSTEN, W. G. & AUSTEN, K. F. 1971 Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. *J Exp Med* 134, 1365-1383.
- RAISZ, L. O. 1976 Mechanisms of bone resorption. In: *Handbook of physiology* vol. 7, sect. 7 (ed G. B. Aurbach), pp. 117-136. American Physiology Society, Washington D.C.
- RASMUSSEN, H. & BORDIER, P. 1974 The physiological and cellular basis of metabolic bone disease. *Williams and Wilkins, Contemporary Biochemistry*.
- SELLINGER, O. Z., BEAUFAY, H., JACQUES, P., DOYEN, A. & DEDUVE, C. 1960 Tissue fractionation studies. 15. Intracellular distribution and properties of β-N-acetyl-glucosaminidase and β-galactosidase in rat liver. *Biochem J* 74, 450-456.
- VAES, G. 1968a Parathyroid hormone-like action of N²,2'-dibutyryl-adenosine-3',5' (cyclic)-monophosphate on bone explants in tissue culture. *Nature* 219, 939-940.
- VAES, G. 1968b On the mechanisms of bone resorption. The action of parathyroid hormone on the excretion and synthesis of lysosomal enzymes and on the extracellular release of acid by bone cells. *J Cell Biol* 39, 679-697.
- VAES, G. 1969 Lysosomes and the cellular physiology of bone resorption. I. Lysosomes in biology and pathology (ed J. T. Dingle and H. B. Fell), pp. 217-253. North-Holland Publishing Company, London.

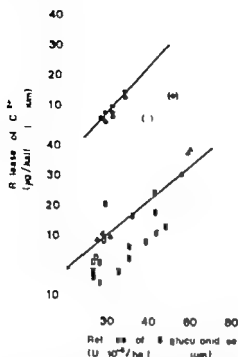


Fig. 4 Correlation between the release of β -glucuronidase and bone resorption evaluated by the release of calcium from cultured calvarial bones. The explants were cultured for 96 hours either without (open symbols) or with 5×10^{-6} M dbcAMP (filled symbols). Data in the upper figure are from the initial 24 h of culture ($r = 0.85$, $P < 0.001$). Data in the lower figure are from the 3rd (Δ , \blacktriangle) and 4th (\square , \blacksquare) day of culture ($r = 0.82$, $P < 0.001$).

lysosomal enzymes LDH and ALAT was uninfluenced. As demonstrated in Fig. 4 there is a significant correlation between bone resorption estimated as the release of Ca^{2+} and release of β -glucuronidase both as regards the inhibitory and stimulatory effect of dbcAMP. These data support the concept of Raisz (1976) that although the specific roles for several lysosomal enzymes released during bone resorption has not been defined the breakdown of bone matrix material probably requires such enzyme activities. With regard to the degradation of collagen which is the major organic component the current opinion seems to be that in most tissues the initial stage involves an extracellular degradation by means of neutral collagenases (Harri & Cartwright 1977). However in contrast there is some controversy about the further breakdown whether it is a mainly extra- or intracellular process. One proposal is that after the primary collagenolysis the second attack is done either by extracellular neutral proteinase or by intracellular lysosomal enzymes (Harri & Cartwright 1977). Another opinion is that the lysosomal enzymes are

released to the extracellular milieu and then contribute to the breakdown of collagen (Van der Pluijm & Lian 1980). Although there seems to be an intimate association between bone resorption and lysosomal enzymes, data from the present and earlier experiments (Vaes 1968a, Vaes 1968b, Eiken & Lian 1971, Doty & Schofield 1971) do not provide conclusive evidence that the acid hydrolases act on their effects extracellularly. However the numerous invaginations of the osteoclast ruffled border could very well offer an extracellular environment suitable for the acid lysosomal enzymes, analogous with the conditions associated with matrix degradation as suggested by Lian & Lian (1978, 1979).

Glucose consumption, lactate production and bone resorption

Bone resorption either promoted or inhibited as shown in the present paper or promoted by PGE₂ (Vaes 1968b) seems to be associated with increased lactate production. The present study shows that while dbcAMP initially decreased bone resorption without affecting glucose consumption and lactate production, the delayed bone resorption effect of this cyclic nucleotide was associated with increased glucose consumption and lactate production. As both $1,25(\text{OH})_2\text{D}_3$ (Brown & Neuman 1979) and $1\alpha(\text{OH})\text{D}_3$ (Lerner 1979) have been shown to stimulate bone resorption, increasing glucose consumption and lactate production it seems that agents which increase bone resorption and the level of cAMP (PTH, dbcAMP) concomitantly increase the production of lactate while hydroxylated derivatives of D which do not increase cAMP promote bone resorption without any change in lactate production. It is therefore possible that increased lactate production may contribute to bone resorption, but that it is obviously not an indispensable part of the mechanism.

Our finding in the present report that stimulation of bone resorption by PGE₂ and dbcAMP is associated with increased content of LDH in bone does not necessarily implicate a role for LDH in bone resorption. It is interesting however that LDH by means of ultrastructural histochemistry has been shown to be concentrated in cytoplasmic vacuoles near the ruffled border in the osteoclast (Coleman et al 1976). Thus PGE₂ and dbcAMP may have increased the content of LDH

protein lipase of human postheparin plasma and adipose tissue in relation to physical training

J. MARNIEMI, PEKKA PELTONEN, ILKKA VUORI and
E. HIETANEN

Research Centre of the Social Insurance Institution, Turku,
Department of Physiology, University of Turku, Finland

MARNIEMI J, PELTONEN P, VUORI I & HIETANEN E. Lipoprotein lipase of human postheparin plasma and adipose tissue in relation to physical training. *Acta Physiol Scand* 1980, 110: 131-135. Received 28 Dec. 1979. ISSN 0001-6772. The Rehabilitation Research Centre of the Social Insurance Institution, Turku and Department of Physiology, University of Turku, Finland.

Adipose tissue lipoprotein lipase and postheparin plasma triglyceride lipase activities were measured in 28 men differing in their physical training activity. They were divided into 4 subclasses based on their training intensity. The two most active classes (17 subjects) having regular heavy exercise at least 4 times/week were considered as the activity training group and the other 11 subjects as classes not training regularly, the control group. In postheparin plasma, the lipoprotein lipase activities were not different between the two groups, whereas training subjects had significantly ($P < 0.02$) lower hepatic lipase activities. Adipose tissue lipoprotein lipase activity was in the training group at about 70% higher level on an average than in the control ($P < 0.10$). A significant positive correlation ($r = 0.38$, $P < 0.05$) was obtained between the adipose tissue lipoprotein lipase activity and the level of physical activity. Our data suggest that even moderate inter-group differences in the physical training activity are reflected as measurable alterations in the adipose tissue lipoprotein lipase activity in man.

Key words: Lipoprotein lipase, hepatic lipase, postheparin plasma, adipose tissue, physical training, man.

protein lipase being located in the capillary lumen of adipose and muscle tissue is a key enzyme in the catabolism of chylomicrons and very low density lipoproteins (Näkälä 1953). These activities and furthermore lipase of origin are known to be released into the system by heparin injection both in laboratory and in man (Robinson 1970). These lipolytic enzymes have been recently studied especially by (e.g. Persson 1973, Campbell et al. 1974, Ten et al. 1975, Pykalä et al. 1975, Taskiran et al. 1977, Schwartz & Brunzell 1978) and human familial hypertriglyceridaemia (Persson & Ljunger et al. 1978).

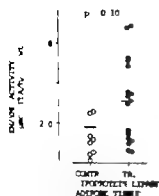
The role of physical exercise as a possible determinant of lipoprotein lipase has gained less attention in laboratory animals, physical training (Katz et al. 1975, Parizkova 1977) and short physical exercise (Näkälä et al. 1963 and 1965, et al. 1977) have been shown to increase the

lipoprotein lipase activity of the skeletal and heart muscle, although also contradictory results have been reported (Askew et al. 1977).

Recently this problem has been investigated also in man in some studies. In champion class long distance runners lipoprotein lipase activity appeared to be considerably higher than in sprinters or control subjects (Näkälä et al. 1978b). In our previous study no immediate response of postheparin plasma lipoprotein lipase to short-term (1 h) exercise was found (Marniemi et al. 1978) which is in accordance with the negligible changes obtained in skeletal muscle lipoprotein lipase activities after the same type of short exercise (Lithell et al. 1979). However in the connection of these short term studies we noticed a tendency towards higher lipoprotein lipase values in well-trained subjects (not published) which encouraged us to carry out the current study.

The purpose of the present study was to investi-

- VAES G & JACQUES P 1965 Studies on bone enzymes. The assay of acid hydrolases and other enzymes in bone tissue. *Biochem J* 97 380-388
- WEISSMAN G, DUKOR P & ZURIER R 1971 Effect of cyclic AMP on release of lysosomal enzymes from phagocytes. *Nature* 231 131-135
- WILLIS J B 1970 Atomic absorption spectrometry. In *Analytical flame spectroscopy* (ed R Mavrodineanu) pp 525-598. Philips Technical Library, Eindhoven 1970
- WRÓBLEWSKI F & LADUE, J S 1951 Lactate dehydrogenase activity in blood. *Proc Soc Exp Biol Med* 90: 210-213
- ZURIER, R. B, WEISSMANN G, HOFFSTEIN V, KAMMERMAN S & TAI H H. 1974 Release of lysosomal enzyme release from human cells. II. Effects of cAMP and cGMP analogues and agents which affect microtubule function. *Invest* 53 297-309



Adipose tissue lipoprotein lipase activities in control subjects (open circles) and trained subjects (black circles). For explanations, see legend in Fig. 1 and Methods.

mean of lipoprotein lipase activities of the control subjects was about 70% higher than in the trained subjects (Fig. 2). Due to the rather marked inter-individual variation, the statistical significance of the difference was low ($P < 0.10$). However, only in one of the control group the enzyme activity was higher than the mean of the training group.

There was a tendency to a positive correlation ($P > 0.10$) between adipose tissue and post-heparin plasma lipoprotein lipase activities in the control group. Calculated from the training or all subjects the correlation was even poorer. Hepatic lipoprotein lipase activity did not correlate significantly with either lipolytic activities measured. Adipose tissue lipoprotein lipase activity appeared to be related to the degree of the training intensity. A significant positive correlation ($r = 0.38$, $P = 0.05$) was found to exist between the enzyme activity and the degree of physical activity expressed by the four training subclasses (Fig. 3).

DISCUSSION

According to the present preliminary data the training effect of physical training on adipose tissue lipoprotein lipase in man is suggested. This accords with the recent results of Nikkila et al. (1978b) who found with champion class long distance runners higher values. Because of the marked inter-individual variation, statistically only a tendency to higher values was found in the present study. These results have to be confirmed by studying the effect of a controlled training program in pre-

viously untrained subjects. These studies are in our next research program.

In post-heparin plasma the relation between lipoprotein lipase activity and the level of physical training was not demonstrable. However, lipoprotein lipase activity in post-heparin plasma originates from several tissue pools, such as adipose tissue and skeletal muscle (Robinson 1970). Accordingly, a very poor correlation was obtained between post-heparin plasma and adipose tissue lipoprotein lipase activities, which is in agreement with earlier reports (Guy-Grand & Berger 1975; Taskiran & Nikkila 1977). All these data stress the preference of using tissue biopsies rather than post-heparin plasma in evaluating lipoprotein lipase activity *in vivo*.

The differences in adipose tissue lipoprotein lipase activities between the groups cannot be explained by different relative weights of the subjects, because this enzyme activity is known to be independent on the degree of obesity when calculated on weight unit basis (Pykalainen et al. 1975; Taskiran & Nikkila 1977; Litbell et al. 1978).

It is of interest in this connection that a positive correlation between adipose tissue lipoprotein lipase activity and plasma HDL-cholesterol concentration has been reported in recent studies of Nikkila's group (Nikkila et al. 1978a and 1978b; Taskiran & Nikkila 1979). In addition, persons with high physical activity at work (Lehtonen & Viikari 1978) or in leisure time (e.g. Carlson & Mossfeldt 1964; Wood et al. 1976) are known to have elevated HDL-cholesterol levels in the circulation and high

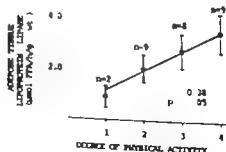


Fig. 3 Correlation between adipose tissue lipoprotein lipase activities and the degree of physical activity expressed as the training class. Indicated are the means of the enzyme activities and their standard errors and the number of subjects (n) in each class. The correlation coefficient (r) and the equation of the curve were calculated by linear regression analysis. For more details about the training classes, see Methods.

Table 1 Ages and body characteristics of actively training and non-training control subjects. Values are indicated as means \pm S.E. The relative body weight was calculated from Geigy Tables. For details classification of the groups see Materials and Methods

	n	Age (yr)	Height (cm)	Weight (kg)	Relative body weight (%)
Controls	11	29.0 \pm 1.2	178.0 \pm 11	78.5 \pm 2.9	103.6 \pm 3.9
Training	17	33.5 \pm 1.4	179 \pm 1.3	72.4 \pm 1.1	92.7 \pm 2.0

gate the role of physical fitness in lipoprotein lipase activity by measuring the enzyme activity in regularly training subjects differing in the intensity of the training. This was accomplished in order to find out if small differences in the physical activity are reflected in lipolytic activity levels.

MATERIALS AND METHODS

Test subjects. 28 healthy men without having any known disorders of fat metabolism were given a detailed questionnaire about their physical training activity. Based on their answers the subjects were divided into 4 subgroups. The groups were as follows: the most active subclass (number 4) was training or competing in heavy physical exercises like running, swimming, cross-country, skiing, soccer etc. regularly at least 4 times a week for the last 12 months or more; the next subgroup (number 3) was having these exercises at least 3 times a week; the following class (number 2) was having light exercise like walking, garden work etc. at least 4 h a week; and the last subclass (number 1) consisted of subjects having clearly less physical exercise than the preceding group. The two most active subclasses were regarded as the actively training group (17 men) and the two less active classes as the non-training control group (11 men). The mean ages and body characteristics of the test subjects in both groups are shown in Table 1. In spite of the higher relative weight in the control group only two subjects in this group could be regarded as obese (relative weight >110% according to Geigy Tables).

Sample and chemical analysis. Postheparin plasma was obtained from the antecubital vein of sitting subjects 10 min after an i.v. injection of heparin (50 IU/kg). This heparin dose is optimal for the release of hepatic lipase (Huttunen et al. 1975b) and compared with clearly higher and more risky doses (100–200 IU/kg) it gives a fairly representative release (70–80%) of the endothelially bound lipoprotein lipase (Krauss et al. 1974; Sauer et al. 1978). The samples were immediately cooled, centrifuged at +4°C and stored at -70°C as most one month until analyzed. Lipoprotein lipase and hepatic lipase activities were measured by the specific immunochemical method of Huttunen et al. (1975b). Specific antiserum against hepatic lipase was a generous gift from Dr Christian Ehnholm, Helsinki, Finland. Adipose tissue specimens (about 100 mg wet weight) were taken as needle (i.d. diameter

2 mm) biopsies from the abdominal region into physiological saline solution and stored intact at -20°C months before enzyme analyses. Their lipase activities were determined according to the method of Hietanen & Greenwood (1977).

The statistical significances of the differences between the groups were assessed by the two-tailed *t*-test.

RESULTS

The individual activities of postheparin lipoprotein lipase and hepatic lipase are presented in Fig. 1. Lipoprotein lipase activities of the subjects were not significantly different from those of the control group. On the other hand, hepatic lipase activity of postheparin plasma was significantly higher (46% on an average, $p < 0.05$) in trained controls than in the actively training subjects (Fig. 1). In the adipose tissue instead

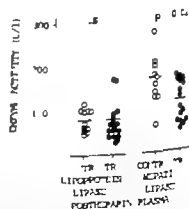
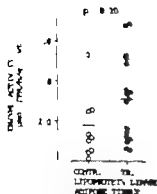


Fig. 1 Postheparin plasma lipoprotein lipase and hepatic lipase activities in controls (open circles) and training subjects (black circles). The means of the actively training group are indicated by horizontal lines. Enzyme activities are expressed units/litre (U/l), where one unit is one μ mole fatty acid liberated/minute. The chances of the differences in enzyme activities between two groups are also given (N.S. = not significant; detail see Methods).



Adipose tissue lipoprotein lipase activities in control subjects (open circles) and trained subjects (black circles). For explanations, see legend in Fig. 1 and Methods.

mean of lipoprotein lipase activities of the subjects was about 70% higher than in the control group (Fig. 2). Due to the rather marked inter-individual variation, the statistical significance of the difference was low ($P < 0.10$). However, only in one of the control group the enzyme activity was higher than the mean of the training group. There was a tendency to a positive correlation ($P > 0.10$) between adipose tissue and post-heparin plasma lipoprotein lipase activities in the training group. Calculated from the training or all subjects the correlation was even poorer. Hepatic lipase activity did not correlate significantly with either lipolytic activities measured. Adipose tissue lipoprotein lipase activity appeared to be related to the degree of the training. A significant positive correlation ($r = 0.38$, $P < 0.05$) was found in excess between the enzyme activity and the degree of physical activity expressed by the four training subclasses (Fig. 3).

DISCUSSION

According to the present preliminary data the effect of physical training on adipose tissue lipoprotein lipase in man is suggested. This accords with the recent results of Nikkila et al. (1977b) who found with champion class long distance runners a marked increase in lipoprotein lipase activity. Because of the marked inter-individual variation, statistically only a tendency to higher values was found in the present study. The results have to be confirmed by studying the effect of a controlled training program in pre-

viously untrained subjects. These studies are in our next research program.

In postheparin plasma the relation between lipoprotein lipase activity and the level of physical training was not demonstrable. However, lipoprotein lipase activity in postheparin plasma originates from several tissue pools such as adipose tissue and skeletal muscle (Robinson 1970). Accordingly a very poor correlation was obtained between post-heparin plasma and adipose tissue lipoprotein lipase activities, which is in agreement with earlier reports (Guy-Grand & Bigot 1975; Taskiran & Nikkila 1977). All these data stress the preference of using tissue biopsies rather than postheparin plasma in evaluating lipoprotein lipase activity in vivo.

The differences in adipose tissue lipoprotein lipase activities between the groups cannot be explained by different relative weights of the subjects, because this enzyme activity is known to be independent on the degree of obesity when calculated on weight unit basis (Pykalainen et al. 1975; Taskiran & Nikkila 1977; Lihell et al. 1978).

It is of interest in this connection that a positive correlation between adipose tissue lipoprotein lipase activity and plasma HDL-cholesterol concentration has been reported in recent studies of Nikkila's group (Nikkila et al. 1978a and 1978b; Taskiran & Nikkila 1979). In addition persons with high physical activity at work (Lehtonen & Viikari 1978) or in leisure time (e.g. Carlson & Mossfeldt 1964; Wood et al. 1976) are known to have elevated HDL-cholesterol levels in the circulation, and high

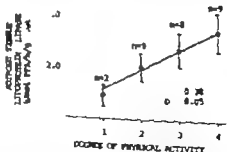


Fig. 3. Correlation between adipose tissue lipoprotein lipase activities and the degree of physical activity expressed as the training class. Indicated are the means of the enzyme activities and their standard errors and the number of subjects (n) in each class. The correlation coefficient (r) and the equation of the curve were calculated by linear regression analysis. For more details about the training classes, see Methods.

Table 1 Ages and body characteristics of actively training and non training control subjects

Values are indicated as means \pm S.E. The relative body weight was calculated from Gey's Tables. For a classification of the groups see Materials and Methods

	n	Age (yr)	Height (cm)	Weight (kg)	Relative body weight (%)
Controls	11	29.0 \pm 1.2	178.0 \pm 2.0	78.5 \pm 2.9	103.6 \pm 3.9
Training	17	33.5 \pm 1.4	179 \pm 1.3	77.4 \pm 2.1	92.7 \pm 2.0

gate the role of physical fitness in lipoprotein lipase activity by measuring the enzyme activity in regularly training subjects differing in the intensity of the training. This was accomplished in order to find out if small differences in the physical activity are reflected in lipolytic activity levels.

MATERIALS AND METHODS

Test subjects. 28 healthy men without having any known disorders of fat metabolism were given a detailed questionnaire about their physical training activity. Based on their answers the subjects were divided into 4 subgroups. The groups were as follows: the most active subclass (number 4) was training or competing in heavy physical exercises like running, swimming, cross-country, skiing, soccer etc. regularly at least 4 times a week for the last 1 month or more; the next subgroup (number 3) was having these exercises at least 3 h a week; the following class (number 2) was having light exercise like walking, garden work etc. at least 4 h a week; and the last subclass (number 1) consisted of subjects having clearly less physical exercise than the preceding group. The two most active subclasses were regarded as the actively training group (17 men) and the two less active classes as the non-training control group (11 men). The mean ages and body characteristics of the test subjects in both groups are shown in Table 1. In spite of the higher relative weight in the control group only two subjects in this group could be regarded as obese (relative weight $>110\%$ according to Gey's Tables).

Sampling and heparin analysis. Postheparin plasma was obtained from the antecubital vein of sitting subjects 10 min after an i.v. injection of heparin (50 IU/kg). This heparin dose is optimal for the release of hepatic lipase (Huttunen et al. 1975b) and compared with clearly higher and more risky doses (100–200 IU/kg) it gives fairly representative release (70–80%) of the endothelially bound lipoprotein lipase (Krauss et al. 1974; Saar et al. 1978). The samples were immediately cooled, centrifuged at $+4^{\circ}\text{C}$ and stored at -20°C at most one month until analyzed. Lipoprotein lipase and hepatic lipase activities were measured by the specific immunochemical method of Huttunen et al. (1975b). Specific antiserum against hepatic lipase was a generous gift from Dr Christian Ehnholm, Helsinki, Finland. Adipose tissue specimens about 100 mg wet weight) were taken as needle (1 d diameter

mm) biopsies from the abdominal region into physiological saline solution and stored intact at -20°C 6 months before enzyme analysis. Their lipolytic activities were determined according to the method of Hietanen & Greenwood (1977).

The statistical significances of the differences between the groups were assessed by the two-tailed *t*-test.

RESULTS

The individual activities of postheparin lipoprotein lipase and hepatic lipase are presented in Fig. 1. Lipoprotein lipase activities of the subjects were not significantly different from those of the control group. On the other hand, hepatic lipase activity of postheparin plasma was significantly higher (46% on an average, $p < 0.01$) in the trained controls than in the actively training subjects (Fig. 1). In the adipose tissue, intracellular

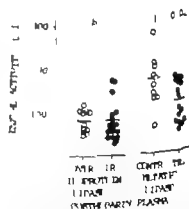


Fig. 1 Postheparin plasma lipoprotein lipase and hepatic lipase activities in controls (open circles) and training subjects (black circles). The means of the activities of the groups are indicated by horizontal lines. Enzyme activities are expressed as $\mu\text{U/litre (U/l)}$ where one μU is one $\mu\text{mole fatty acid liberated/minute}$. The chances of the differences in enzyme activities between the two groups are also given (N.S. = not significant, details see Method).

- in normal and pathological sera and the effect of
 use on serum lipoproteins. *Scand J Clin Lab Invest*
 suppl 12: 1-101.
- LÄ, E. A., TORSTI P. & PENTTILÄ, O. 1963
 effect of exercise on lipoprotein lipase of rat heart,
 one tissue and skeletal muscle. *Metabolism*
 363-365.
- LÄ, E. A., TORSTI P. & PENTTILÄ, O. 1965
 effects of fasting, exercise and resynthesis on
 cholinergic control and lipoprotein lipase activity
 at heart and adipose tissues. *Life Sci* 4: 27-35.
- LÄ, E. A., TASKINEN M.-R. & KEKKI M.
 1976 Relation of plasma high-density lipoprotein
 cholesterol to lipoprotein-lipase activity in adipose tis-
 sue and skeletal muscle of man. *Atherosclerosis*
 87-90.
- LÄ, E. A., TASKINEN M.-R., REHUNEN S.
 & LARKÖNEN M. 1978a Lipoprotein lipase activity
 in adipose tissue and skeletal muscle of runners.
 in serum lipoproteins. *Metabolism* 27: 1661.
- KOIVA, J. 1977 Metabolic activity of adipose tis-
 sue and fatty acid oxidation during adaptation to dif-
 ferent loads. In: *Body fat and physical fitness* (ed. J.
 Koiva), pp. 90-95. Martinius Nijhoff B. V. Medical
 Science, The Hague.
- GON, B. 1973 Lipoprotein lipase activity of human
 adipose tissue in different types of hyperlipidaemia.
Med Scand 193: 447-456.
- PYKÄLISTÖ O. J., SMITH P. H. & BRUNZELL, J.
 D. 1975 Determinant of human adipose tissue lipopro-
 tein lipase. Effect of diabetes and obesity on basal
 and diet-induced activity. *J Clin Invest* 67: 1108-1117.
- ROBINSON D. S. 1970 The function of the plasma tri-
 glycerides in fatty acid transport. In: *Comprehensive*
biochemistry (ed. M. Florkin and E. H. Stoltz) vol. 11
 pp. 51-116. Elsevier, Amsterdam.
- SAUJAR J., SKREDE, S. & BLOMHOFF J. P. 1978
 Hepatic lipase and lipoprotein lipase in posthepatic
 plasma in liver disease. Relations to plasma proteins.
Clin Chim Acta 84: 13-23.
- SCHWARTZ, R. S. & BRUNZELL, J. D. 1978 In-
 creased adipose-tissue lipoprotein-lipase activity in
 moderately obese men after weight reduction. *Lancet*
 i: 1230-1231.
- TASKINEN M.-R. & NIKKILÄ, E. A. 1977 Lipo-
 protein lipase activity in adipose tissue and in post
 hepatic plasma in human obesity. *Acta Med Scand*
 202: 399-408.
- TASKINEN M.-R. & NIKKILÄ, E. A. 1979 Effects
 of caloric restriction on lipid metabolism in man.
Atherosclerosis 32: 289-299.
- WOOD P. D., HASKELL, W., KLEIN H. LEWIS S.
 & STERN M. P. & FARGUHAN, J. 1976 The distribu-
 tion of plasma lipoproteins in middle aged runners.
Metabolism 25: 149-157.

HDL-cholesterol concentrations are generally considered protecting against coronary heart disease (cf Nikkila 1953 Gordon et al 1977). Thus by increasing the lipoprotein lipase activity by physical training there might be a possibility in this way to accelerate the favourable removal of triglycerides from the circulation connected to the increment of HDL-cholesterol.

However the exact mechanism between HDL-cholesterol and lipoprotein lipase if any is still unknown and more detailed studies have to be done to ascertain it. The importance of lipoprotein lipase as a regulator of triglyceride metabolism is further emphasized by the finding that lipoprotein lipase activity is the only metabolic abnormality associated with obesity that does not become normal after weight reduction (Schwartz & Brunzell 1978).

The physiological role of hepatic lipase is not yet fully understood. According to a recent study by Kuusi et al (1979) hepatic lipase might be involved in the uptake of LDL and HDL-cholesterol into the liver causing perhaps an increase in the densities of these particles. Thus a shift from HDL₂-subfraction to more dense HDL₃-subfraction could occur due to the action of this lipase. It has been suggested that HDL₂-subfraction is a better predictor of the development of coronary heart disease (Miller & Miller 1978). The decreased hepatic lipase activity in the training group obtained in this study could thus have a beneficial effect on the distribution of the two HDL subfractions. However the clarification of the possible relationship between hepatic lipase and physical activity awaits for the future.

This study was financially supported by the Yrjö Jahnsson Foundation, Finland.

REFERENCES

- ASKEW E, W DOHM G L, HUSTON R L, SNEED T W & DOWDY R P 1972. Response of rat tissue lipases to physical training and exercise. *Proc Soc Exptl Biol Med* 141: 123-129.
- BORENSZTAJN J, RONE M B, BABIRAK S P, McGARR J A & OSCAL L B 1975. Effect of exercise on lipoprotein lipase in rat heart and skeletal muscle. *Amer J Physiol* 229: 394-397.
- CAMPBELL R G, FAJISOFF B & BRODOWS R E 1974. Adipose tissue lipoprotein lipase activity in obesity. *Diabetes Suppl* 1: 367.
- CARLSON L A & MOSSFELDT F 1964. Acute effects of prolonged heavy exercise on the concentration of plasma lipids and lipoproteins in man. *Acta Physiol Scand* 62: 51-59.
- GORDON R, CASTELLI W P, HORTLAN C, KANNEL W B & DAWBER T R 1977. Density lipoprotein as a protective factor against coronary heart disease—The Framingham Study. *Med* 62: 707-714.
- GUY-GRAND B & BIGORIE B 1975. Effect of size-restrictive diet and diabetes on lipoprotein release by human adipose tissue. *Horm Metab* 7: 471-475.
- HIETANEN E & GREENWOOD M R C 1976. Comparison of lipoprotein lipase activity and differentiation in growing male rats. *J Lip* 18: 480-490.
- HUTTUNEN J & EHNHOLM C, NIKKILA E & OHTA M 1975a. Effect of fasting on lipoprotein lipase and hepatic lipase and removal in obese subjects. *Europ J Clin Invest* 4: 445.
- HUTTUNEN J & EHNHOLM C, KOSKINEN J & NIKKILA E A 1975b. A new method for the selective measurement of lipoprotein lipase in human postheparin plasma. *Chim Acta* 63: 335-347.
- HUTTUNEN J & EHNHOLM C, KOSKINEN J & NIKKILA E A 1976. Postheparin plasma lipase and hepatic lipase in normal subjects and patients with triglyceridaemia: correlations to various parameters of triglyceride metabolism. *Clin Sci Mol Med* 50: 249-260.
- KRAUSS R M, LEVY R I & FREDRICKSON D S 1974. Selective measurement of two lipoprotein lipases in postheparin plasma from normal subjects and patients with hyperlipoproteinaemia. *J Clin Invest* 54: 1107-1124.
- KUUSI T, KINNUNEN P K J & NIKKILA E 1979. Hepatic, endothelial lipase and serum rat plasma low and high density lipoproteins. *FEBS Lett* 104: 384-388.
- LEHTONEN A & VIKARI J 1978. The effect of vigorous physical activity on work on serum lipids: a special reference to serum high-density lipoprotein. *Acta Physiol Scand* 104: 117-121.
- LITHELL H, BOBERG J, HELLSING K, WAERN U 1978. Lipoprotein-lipase activity in cutaneous adipose tissue in healthy subjects. *J Clin Med Sci* 83: 45-5.
- LITHELL H, HELLSING K, LUNDQVIST M, MALMBERG P 1979. Lipoprotein lipase activity in human skeletal muscle and adipose tissue after active physical exercise. *Acta Physiol Scand* 113: 315.
- MARNIEMI J, PELTONEN P, VUORI E, HIETANEN E 1978. Triglyceride lipase activity in human post-heparin plasma in obese and normal subjects in physical exercise. *Acta Physiol Scand* 104: 38A-40A.
- MILLER G J & MILLER N E. *Diet, high density lipoprotein and coronary heart disease*. High density lipoproteins and atherosclerosis. F M Otto Jr, N E Miller and M F Oliver (eds). 95-107. Elsevier, Amsterdam 1978.
- NIKKILA E 1953. Studies on the lipid-protein metabolism.

-ganglionic cholinergic excitatory pathways to sympathetic supply to the feline stomach parent system or afferents with excitatory axon collaterals?

DELBRO and B. LISANDER

Department of Physiology, University of Göteborg, Sweden

DELBRO D. & LISANDER, B. Non-ganglionic cholinergic excitatory pathways in the sympathetic supply to the feline stomach—axonal afferents or afferents with excitatory axon collaterals. *Acta Physiol Scand* 1980, 110: 137-144. Received 28 Dec. 1979. ISSN 0001-6772. Department of Physiology, University of Göteborg, Sweden.

Experiments were performed on chloralosed, adrenalectomized cats, paralyzed with gallamine and artificially ventilated. Gastric motility was recorded by the balloon method. Efferent stimulation of the cat greater splanchnic nerve, well proximal to the celiac ganglion, could either increase or decrease gastric tone. The excitatory responses called for longer stimulation intervals than the inhibitory ones but were as a rule observed at lower frequencies only (1-4 Hz). They could be abolished by atropine but were not prevented by bilateral vagotomy, hexamethonium nor guanethidine. The latter two drugs rather reversed inhibitory responses to excitatory ones which exhibited a hyperbolic frequency-response relationship with maximal effect already at 2-4 Hz. Heating of nerve trunk selectively activates the afferents of the delta group and C-fibres. Heating of the greater splanchnic nerve caused an increase in gastric motility which like that caused by electric stimulation was not prevented by hexamethonium nor guanethidine nor was it eliminated by cutting the nerve centrally nor by vagotomy, like it was abolished by atropine. These results suggest that the excitatory gastric responses to efferent splanchnic nerve stimulation are due to antidromic activation of the afferent fibres. Their functional significance remains obscure but their peripheral arborizations may convey non-reflexes influencing gastrointestinal motility.

Key words: Gastric excitation, sympathetic, atropine sensitive, axon reflexes.

rent splanchnic nerve stimulation can decrease gastric tone by an activation of the adrenergic fibres (Bass & Costa 1974). However, atropine sensitized excitatory responses have also been recorded (cf. Thomas & Baldwin 1968) and in the present series of experiments these less studied inhibitory effects were further analysed. A preliminary report has been given (Delbro & Lisander 1979).

METHODS

Adult weighing 1.95-6.25 kg were used. The animals were deprived of food 24-36 h before the experiments. After anaesthesia with ether they were anesthetized with xylazine 50 mg/kg b.w.t. A tracheal cannula was tied. A 1% infusion (0.10-0.15 ml/min) of 10-20 mEq HCO₃/100 ml, dissolved in 10% glucose solution, was used to counteract acidosis (Haglund 1973). After midline

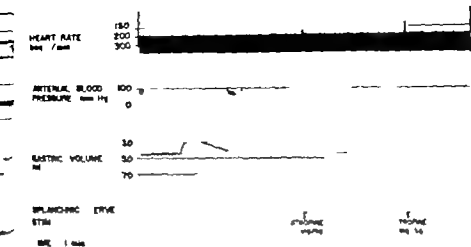
laparotomy the adrenals were ligated to eliminate the release of catecholamines to the blood stream. Temperature was kept at 37-38°C by means of heating pad.

Recording of effector responses

Gastric volume was in most experiments recorded with a large rubber balloon introduced via the esophagus (cf. Jansson 1969). Intragastric pressure was in the individual experiments kept constant at 5-17 cm H₂O. The system was filled with body warm water and the balloon was connected to a container placed on a Statham strain gauge recording the weight of the water outside the stomach. In this way changes of gastric volume could be continuously followed at largely constant transmural pressure.

In a few experiments the volume of the stomach was recorded by pyloric cannula (Jansson & Martinsson 1966).

Blood pressure was recorded by Statham P23 AC transducer connected to catheter in femoral artery. Heart rate was in some experiments recorded on Grass tachograph unit, triggered by the arterial pulse wave. All recordings were made on Grass polygraph.



Continuation from Fig. 1. The excitatory gastric response to splanchnic stimulation is considerably reduced by 0.1 mg/kg. and is completely abolished by 1 mg/kg.

used by submaximal efferent vagal stimulation (5–10 V), excitatory responses could still be elicited by splanchnic nerve stimulation (4 expts.).

was also the case if gastric relaxation first induced by activation of the vagal high-threshold non-adrenergic non-cholinergic fibres (2.0 V) (expt. of Jansson 1969).

Use of drugs

On 4 expts. the muscle relaxant, gallamine 4 g. was repeatedly given. However also prior to drug excitatory (6 expts.) as well as inhibitory (7 expts.) gastric responses could be elicited by efferent splanchnic stimulation.

Guanethidine, 3.3–20 mg/kg. abolished the inhibitory responses to splanchnic nerve stimulation (5 expts.) but did not counteract the excitatory ones. It was clear from the fact that this drug did not abolish the responses in animals where the inhibitory ones had been abolished by hexamethonium (7 expts. see Fig. 1).

Hexamethonium, 5–100 mg/kg. abolished or partly diminished the inhibitory responses (7 expts.). The drug could in this way unmask excitatory responses. If given after guanethidine it did not diminish the excitatory effects (4 expts.). In one experiment, the ganglionic blocker trimethaphan 1 mg/kg. also abolished the inhibitory splanchnic responses and revealed excitatory ones. After hexamethonium and guanethidine given in combination (13 expts.) or separately only excitatory

responses were observed and then at higher stimulation frequencies as well (see below).

One cat was pretreated with reserpene 3.8 mg/kg. a.c. 24 h prior to the expt. In this animal excitatory effects only were observed upon splanchnic

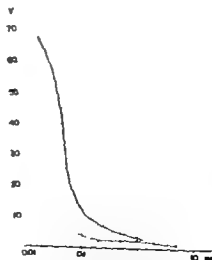


Fig. 3. Cat 2.45 kg. Threshold intensities to elicit inhibitory (dashed line) and excitatory (solid line) gastric responses by efferent left splanchnic stimulation (10 Hz). The excitatory responses were obtained after guanethidine and hexamethonium (both at 4 mg/kg). At each pulse duration one subthreshold and one supra-threshold voltage was determined, as indicated in the figure.

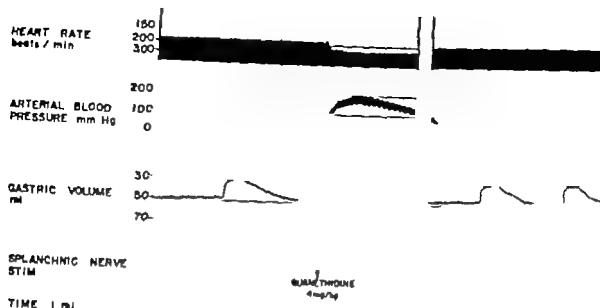


Fig. 1 Cat 5 kg treated with hexamethonium 5 mg/kg. Efferent left splanchnic nerve stimulation (4 Hz) before and after guanethidine 4 mg/kg. The time between the panels is about 70 min.

Nerve stimulation

The vagi were usually dissected free in the neck and put on strings so that they could be cut in the course of the experiments. Efferent vagal stimulation was accomplished by ring electrodes slipped over the cut nerve ends. The greater splanchnic nerve, mostly on the left side, was carefully dissected free, usually in the abdomen, centrally cut and the distal end arranged for efferent stimulation with a ring electrode. Care was taken to keep the electrode site at least 10 mm proximal to the celiac ganglion. Nerve stimulations were performed with a Grass S5 stimulator or a specially constructed stimulator both delivering unidirectional rectangular pulses.

Graded heating of the greater splanchnic nerve was performed by circulating warm water through a metal block with a groove containing the nerve. Metal blocks of different widths, viz. 3.6 and 8 mm, were used. The warm water was heated in a thermostated bath and delivered by a circulation pump. The temperature of the metal block was recorded on the Grass polygraph by a thermometer. One of the heating units also contained electrodes for electrical stimulation of the same nerve section. The splanchnic nerve to be heated was cautiously dissected free but was either left intact or cut proximally. When the greater splanchnic nerve was to be dissected in the chest the thorax was opened laterally by resection of two or more ribs.

Drugs

In most experiments, artificial respiration was maintained with a respiration pump and gallamine triethiodide (Flaxedil, May & Baker) was repeatedly given to prevent neuromuscular interference. Other drugs used were atropine (atropine sulphate, Merck), hexamethonium (hexamethonium chloride, Fluka), eserine (physostigmine salicylate, Merck), trimethaphan (Arfonad, Roche), all dissolved in isotonic saline, further guanethidine (Lamelin

CIBA), hydrocortisone (Solu-Cortef, Upjohn, U.S.A.), (Naloxone, Endo) and reserpine (Serpasil, CIBA). All are expressed in terms of the salts and were used as such except for reserpine which was injected as the base and hydrocortisone which was given as

RESULTS

Inhibitory effects of splanchnic nerve stimulation

Efferent electrical stimulation of the left splanchnic nerve regularly decreased gastric tone. In the vagally decentralized stomach the latent onset was more than 10 s. This was also the case when splanchnic stimulation was performed against a background of vagally induced increase in gastric tone.

Excitatory effects of splanchnic nerve stimulation

Efferent splanchnic nerve stimulation could increase gastric tone provided that the stimulus strength was high enough (see below) & treatment with autonomic blocking drugs. Excitatory responses were most pronounced at stimulation rates below 8 Hz. Their latency of onset was about the same as that of the inhibitory responses and not seldom the changes in gastric tone were biphasic with excitation preceding inhibition. Excitatory responses were not prevented by vagotomy in the neck (8 experiments). If gastric tone

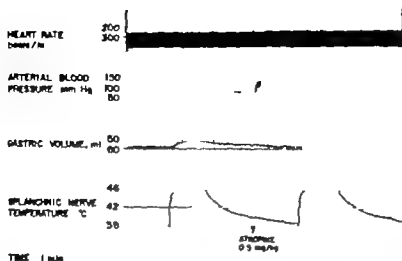


Fig. 5 Cat 3.25 kg, treated with guanethidine 3.4 mg/kg and hexamethonium, 20 mg/kg and bilaterally vagotomized. Heating of the intact left splanchnic nerve in the abdomen causes gastric excitatory response which is prevented by atropine 0.5 mg/kg.

or the frequencies that led to a maximal rate (2-4 Hz). At still higher stimulation frequencies the rate of increase in gastric tone was probably more rapid. It was noted throughout that the excitatory responses to splanchnic stimulation could be kept quite stable during stimulations at 4 Hz or below for up to 1 min. At 8 Hz or above there was, however, an 'escape' in the response, more pronounced the higher the frequency. Furthermore, it was often observed that stimulation at 8 Hz or above could decrease the magnitude of the response in a subsequent stimulation. Repeated stimulations at high frequencies considerably diminish the subsequent excitatory responses.

Effects of heating of the splanchnic nerve

Heating of a nerve selectively activates afferent fibres of the A delta group and C class (Baker 1947). It was considered of interest to study the effects of heating of the splanchnic nerve to decide whether the excitatory splanchnic effect might be due to an antidromic activation of efferent fibres. Heating of the splanchnic nerve in the abdomen or in the thorax, regularly and reproducibly (7 expts.) elicited an increase in gastric tone. Temperatures up to 46°C were required. The responses occurred with a fairly short latency of onset (see Fig. 4). Like the excitatory responses to

electric splanchnic nerve stimulation they were neither prevented by vagotomy in the neck (5 expts.), nor by guanethidine 3.3-10 mg/kg (3 expts.), nor hexamethonium 5-10 mg/kg (4 expts.), nor by these drugs in combination (5 expts.). Atropine however abolished these responses at the same dose level which blocked the electrically induced excitatory responses 0.1-1.0 mg/kg (5 expts.) (Fig. 5).

In 4 expts. the splanchnic nerve was left with intact central connections. Then heating of the nerve elicited signs of reflex activation of sympathetic efferents. For example pressor responses regularly occurred which could be essentially blocked by guanethidine 3.4-4 mg/kg and hexamethonium, 5 mg/kg (7 expts.). Administration of these drugs enhanced however excitatory gastric responses to heating of the intact nerve (3 expts.). In no case were gastric inhibitory responses induced by heating of the nerve when reflex effects had been prevented by cutting the nerve centrally indicating that heating never activated efferent inhibitory pathways directly. On the other hand, prior to the administration of autonomic blocking drugs and vagotomy heating of the intact nerve did not elicit gastric contraction. Instead there was often a tendency towards gastric relaxation. Cutting the vagi abolished the slight relaxatory responses to heating and reversed them to contractions (3 expts.).

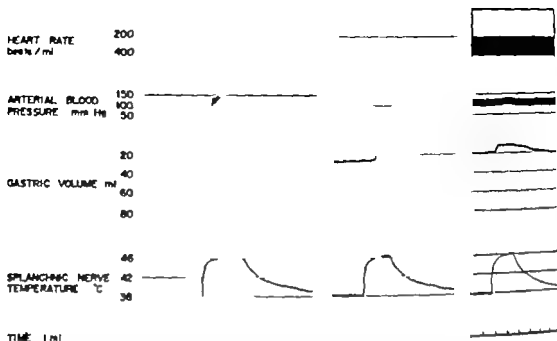


Fig. 4. Cat, 8 kg. Heating of the intact left splanchnic nerve in the abdomen. First panel: The heating causes response as long as the vagi are intact. Second panel: After bilateral vagotomy in the neck, heating does not excite. Third panel: After hexamethonium, 5 mg/kg, splanchnic heating still causes gastric excitation but previously observed reflexpressor responses are virtually abolished.

nerve stimulation ($2-3^{\circ}$ Hz, 1 ms, 10 V). Atropine at a dose of 0.1–1 mg/kg completely abolished the excitatory effects in all instances (6 expts; see Fig. 2). When this drug was given following guanethidine and hexamethonium no reversal to inhibitory effects to splanchnic stimulation was observed, indicating that the adrenergic effects on gastric motility had previously been fully blocked. In two expts naloxone, 0.015–0.080 mg/kg, was given but had no effect on the excitatory responses following treatment with guanethidine and hexamethonium. Eserine was given in 2 expts, 0.2 and 0.5 mg/kg. An augmentation of the gastric excitatory responses as well as a decrease in the threshold for their elicitation were then noted. In 3 cats treated with hydrocortisone, 5 mg/kg i.m., to compensate for the elimination of adrenocortical secretion, the excitatory gastric responses to splanchnic stimulation were of essentially the same character as in the other expts.

The effects of variation of stimulus intensity

In 4 expts the left splanchnic nerve was stimulated at various intensities before and after administration of both guanethidine and hexamethonium, 5 and 10 mg/kg, respectively. The aim was to establish the threshold intensities needed to elicit inhibitory and excitatory gastric responses. Strength du-

ration curves were drawn on the basis of the observations and Fig. 3 shows the result of one representative expt. The thresholds for the inhibitory responses were about the same, regardless of whether or not the cut vagi were simultaneously stimulated in the neck in order to accentuate the perigastric tone (1 expt). The observations clearly indicated that decidedly higher stimulus rates were required to elicit the sympathetic excitation effects than the inhibitory ones.

Frequency response relationships

In 3 cats given both guanethidine and hexamethonium, 5–20 mg/kg, respectively, the magnitude of the excitatory responses was determined at various frequencies of stimulation (0.5, 1, 2, 5, 10, and 16 Hz). In additional expts the greater splanchnic nerve was stimulated with higher frequencies, 10–30 Hz. Responses could be obtained already at 0.5 Hz in one expt, but in the others effects required at least 1 Hz. When the frequency was increased maximal responses were obtained already at 2–4 Hz and still higher rates were intended to decrease the magnitude of the response. At low frequencies giving submaximal responses there was a slow build-up of tone before a steady contraction level was reached. The same was

by conventional ganglionic blocking. Several explanations to this may be offered. Possible ganglionic synapses in the pathways body may have been located proximal to the tag electrode. Alternatively the phenomena have been due to activation of exceptionally short preganglionic efferents with a transmission resistant to conventional ganglionic drugs. However a third possibility is that the dry responses were due to antidromic activation of afferent fibres. To elucidate the latter possibilities with heating of the splanchnic nerve were performed. Heating of a nerve activates III of the A delta group and C class (Euler 1947). Heating of the splanchnic nerve to 46°C in the abdomen or in the thorax, elicited gastric contractions.

Responses showed the same pharmacological properties as the electrically induced excitations. They were not blocked by guanethidine nor by atropine. The blocking of atropine suggests that these afferent ramify and may release acetylcholine, alternatively that they activate intramural cholinergic neurons via a substance resistant to conventional cholinergic blockers.

Transection of the centrally cut nerve did not activate any gastric fibres. However with the nerve intact there were regularly indications that heating of afferent nerve fibres, producing widespread activation of visceral efferents. For example, relaxation was sometimes observed if the nerves were still intact. Vagotomy reversed relaxatory responses to contractions. After splanchnic nerve stimulation causes a reflex relaxation of the vagal non-adrenergic non-cholinergic relaxatory fibres (Abrahamsson, Glise 1979). The present observations suggest that effect involves activation of thin splanchnic afferents. Furthermore heating of the intact splanchnic nerve regularly induced pressor responses which could be essentially abolished by thidione and hexamethonium. These pressor responses were evidently indications of widespread sympathetic excitation.

In the present experimental evidence suggests that the excitatory gastric responses to anti-sympathetic stimulation are due to anti-sympathetic activation of thin afferent nerve fibres. The fact that these effects are effectively blocked by atropine may possibly indicate that acetylcholine

can be released from afferent neurons. Another alternative is that the afferents act via a noncholinergic nonadrenergic transmitter on intramural cholinergic neurons to elicit the contraction response.

The functional significance of these excitatory gastric effects by afferents is obscure. One possibility is that they are involved in visceral reflexes within the stomach. If so, since that visceral afferents can be activated by noxious stimuli, we suggest that they may play a role for the local pathophysiological manifestations in conditions like gastritis or gastric ulcer.

The present study has been supported by grants from the Swedish Medical Research Council (14X-04249), Bergvall Foundation, Groschansky's Foundation, Jönsson Foundation, the Swedish Society for Medical Research and the Medical Faculty of Göteborg. Thanks are due to Mrs Ann Wikström for excellent technical assistance.

REFERENCES

- ABRAHAMSSON H, GLISE, H & GLISE, K 1979 Reflex suppression of gastric motility during laparotomy and gastroduodenal nociceptive stimulation. *Scand J Gastroenterology* 14 (1) 101-106.
- CELANDER, O & FOLKOW B 1953 The correlation between the stimulation frequency and the dilator response evoked by antidromic excitation of the thin afferent fibres in the dorsal roots. *Acta Physiol Scand* 29: 371-376.
- DELBRO D & LISANDER, B. 1979 Sympathetic atropine sensitive effects on gastric motility. *Acta Physiol Scand* 105: 63 A.
- EULER, C von 1947 Selective responses to thermal stimulation of splanchnic nerves. *Acta Physiol Scand Suppl* 43: 14-173.
- FOLKOW B & NEIL, E. 1971 *Circulation*. Oxford University Press, New York, London, Toronto.
- FURNESS, J B & COSTA M 1974 The adrenergic innervation of the gastrointestinal tract. *Reviews of Physiology* 69: 1-51.
- HAGLUND U 1973 Vascular reactions in the small intestine of the cat during hemorrhage. *Acta Physiol Scand* 89: 129-141.
- JANSSON G 1969 Extrinsic nervous control of gastric motility. An experimental study in the cat. *Acta Physiol Scand Suppl* 326: 1-42.
- JANSSON G & MARTINSON J 1966 Studies on the ganglionic action of action of sympathetic outflow to the stomach. *Acta Physiol Scand* 68: 184-192.
- JANSSON G, LISANDER, B & MARTINSON J 1969 Hypothalamic control of adrenergic outflow to the stomach in the cat. *Acta Physiol Scand* 75: 176-186.

In 2 experiments heating and electrical stimulation were performed on the same part of the centrally cut splanchnic nerve. Although the responses were qualitatively similar, heating did not induce excitatory effects of the same magnitude as those elicited by supramaximal electrical stimulation.

DISCUSSION

Both inhibitory and excitatory gastric responses could be elicited by efferent splanchnic nerve stimulation. In the vagally decentralized stomach inhibitions were slow in onset and not appreciable except at higher rates of stimulation. This is compatible with the concept that the inhibitory adrenergic sympathetic fibres to the gastrointestinal tract exert their main action not on the smooth muscle cells but on intramural excitatory neurons in series with the vagal motor pathways (cf. Furness & Costa 1974). However, the latencies to splanchnic nerve stimulation were considerable even during efferent vagal stimulation, in contrast to the brisk inhibitions occurring during activation of the spinal intestinal gastric reflex (Jansson & Martinson 1966) or hypothalamic stimulation (Jansson, Lisander & Martinson 1969). These observations may perhaps suggest that the direct nerve stimulation activates another fibre population than that involved in reflexly or centrally induced sympathetic inhibitory responses. The interest was here focused on the less studied excitatory responses to splanchnic stimulation. If no autonomic blocking drugs had been given these responses were usually most pronounced at stimulation rates below 8 Hz and at higher frequencies biphasic or purely inhibitory responses were observed. The cholinergic mediation of these excitatory responses was evidenced by the blocking effect of atropine in consonance with findings by others (cf. Thomas & Baldwin 1968). The cholinesterase blocking agent physostigmine increased the responses and lowered their threshold of elicitation.

Further, the excitatory responses were not counteracted by the presynaptically active adrenergic blocking agents guanethidine and reserpine. Rather by abolishing the inhibitory gastric responses these drugs unmasked or further augmented the excitatory responses (see below).

The splanchnic nerve stimulations were always performed well proximal to the celiac ganglion. In spite of this the ganglionic blocking agents

hexamethonium and trimethaphan could not abolish the excitatory responses. The inhibitory responses, however, were completely abolished by these at low dose levels. If hexamethonium was after adrenergic blockade with guanethidine excitatory responses were not diminished.

Thus the pharmacological analysis suggests that the inhibitory responses to direct splanchnic nerve stimulation were due to activated ganglionic fibres, exciting postganglionic adrenergic neurons. The excitatory responses, on the other hand, apparently did not involve postganglionic adrenergic neurons, but rather with conventional ganglionic transmission which could be blocked by the muscarinic blocker atropine.

Stimulation of the splanchnic nerves at intensities established that the inhibitory and excitatory gastric responses were mediated by different fibre populations with clearly different excitation thresholds. Thus lower intensities were sufficient to elicit inhibitory effects, which corresponded with an activation of preganglionic sympathetic fibres. The excitatory effects required considerably higher stimulation intensities and the thresholds agree well with those reported for celiac (Koslow, Bak & Li 1973; Öberg & Thorsén 1974). It should be borne in mind, however, that the responses rather than action potentials have been used as the indicator of nerve fibre activation in the present experiments. Several nerve fibres may need to be activated to induce clear effector responses. The present approach can therefore be expected to give higher threshold values than when single fibre preparations are studied. Furthermore, a considerable source of error might affect the two types of responses to a different degree. Experiments with the aid of recording the action potentials in the splanchnic nerve are in progress to further elucidate this problem.

In cats treated with guanethidine and hexamethonium to block the inhibitory effects, excitatory responses could be elicited at stimulation frequencies as low as 0.5 Hz. At 4 Hz and higher contraction responses were obtained. Such a low range frequency response relationship is seen for the axon reflex vasodilator arrangement related to somatic C fibre afferents (Clandfield & Foy 1953) but is well below that characterizing autonomic neuro-effector systems where the stimulation frequencies usually are around 10–15 Hz (cf. Koslow & Neil 1971).

As mentioned the excitatory effects could be

Convulsants (benzylpenicillin and pentylenetetrazol) on potential clamped myelinated axons from the rat

BRISMAR and J. O. R. JEFFERYS¹

¹Department of Clinical Neurophysiology, Huddinge Hospital, Huddinge, Sweden

BRISMAR, T. & JEFFERYS J. O. R.: Convulsant (benzylpenicillin and pentylenetetrazol) on potential clamped myelinated axons from the rat. *Acta Physiol Scand* 1980; 110: 145-148. Received 16 Jan. 1980. ISSN 0001-6772. Department of Clinical Neurophysiology, Huddinge Hospital, Huddinge, Sweden.

The effect of two convulsants, benzylpenicillin and pentylenetetrazol, was tested in potential clamp experiments on single rat myelinated nerve fibres. 10 mM concentration did not affect the permeability properties of the nodal membrane. The convulsant action is therefore probably not mediated through changes in the excitability of central axons.

Key words: Myelinated nerve potential clamp, benzylpenicillin, pentylenetetrazol and penicillins, epilepsy.

Several mechanisms have been implicated in the action of penicillin (Pc) and of pentylenetetrazol (PTZ). Several lines of evidence point to changes in synaptic transmission. Depressant effects on inhibitory postsynaptic potentials have been reported, for instance in *Aplysia* neurones (2 mM PTZ, Pednar & Wilson 1976, 1977), and also in hippocampal pyramidal cells (0.3-4 mM Pc, Llinás & Qvistad 1980). Pc increases the excitability of presynaptic terminals or myelinated axons in several excitatory projections in mammals (e.g. in various thalamo-cortical paths (Gustafsson 1972, Rosen et al. 1973, Scobey & Schwartzkroin et al. 1974, Noebel & Noebel 1978a, b), in the pyramidal tract (Llano & Noebel 1978), and in the nerve-muscle preparation (2.5-8.5 mM Pc, Noebel & Price 1977)). Studies on hippocampal slices, have indicated that the properties of the non-synaptic membrane have been modified by Pc by involving regenerative calcium currents or (Price & Schwartzkroin 1978, Andersen & Langmoen 1978) and so causing the action potential shift in positive direction which is also those initially described *in vivo* by MacLeod & Aronow-Marian (1964).

The excitability of the postsynaptic spike generation mechanism has been increased by rather low concentrations of each drug. PTZ in the range

of 25-170 mM elicited spontaneous firing in molluscan neurones, which was related to a reduction in the threshold of the initial regenerative inward current of the action potential and to a decrease in the subsequent steady state outward current (Hille, Faber & Hess 1973, David, Wilson & Escueta 1974, Williamson & Crill 1976). In the frog node of Ranvier similar changes caused by low external calcium and added tetraethylammonium respectively also resulted in repetitive firing (Bergman, Nooner & Stämpfli 1968). Application of Pc (34-84 mM) to slices of olfactory cortex was reported to increase postsynaptic excitability specifically (Voskuyl & ter Keurs 1978).

The present study examines the effect of convulsant doses of Pc or PTZ (10 mM each) on the ion currents at the nodes of Ranvier of fibres isolated from rat sciatic nerve.

METHODS

Single myelinated nerve fibres (10-12 µm in diameter) were isolated from the sciatic nerve of albino rats (Sprague-Dawley 250-300 g female). A single fibre was mounted in a recording chamber and connected to the electric feed back system for recording or clamping mem-

On leave from the Royal Free Hospital, London. Present address: Institute of Neurology, The National Hospital, Queen Square, London WC1N 3BG.

- KOSLOW M, BAK A & LI C L. 1973 C-fiber excitability in the cat. *Experimental Neurology* 41: 745-753.
- ÖBERG B & THOREN P. 1973 Circulatory responses to stimulation of medullated and non medullated afferents in the cardiac nerve in the cat. *Acta Physiol Scand* 87: 1-11.
- THOMAS J L & BALDWIN M V. 1968 *Handbook of physiology*. Sect 6 Vol 13 pp. 197-199.

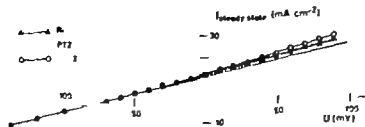


Fig. 3 Steady state membrane current I vs. potential relation in Ringer 10 mM PTZ Ringer and after wash out. Same experiment as in Fig. 2.

permeability increase and the relatively large conductance (Brisman 1980) were unaffected.

DISCUSSION

Investigation has shown that Pc and PTZ, at concentrations considerably greater than those known to induce epileptiform discharges in the brain, have no effect on the potential-dependent activities of myelinated fibres from the sciatic nerve of rat. If as seems likely excitation in central myelinated axons has similar properties, then it is unlikely that they mediate the convulsive actions of PTZ. It is also possible that the action potential mechanism at the trigger zones of central neurones resembles that at the nodes. If that were the case the convulsive effects of the two drugs probably are not due to increased excitability of postsynaptic sites.

The possible action of two widely used convulsants, phenytoin (Pc) and pentylenetetrazol (PTZ), on the nodal membrane was tested in potential clamp experiments on single myelinated nerve fibres from the sciatic nerve of the rat. The specific permeability properties associated with the action for impulse conduction were analysed. Neither Pc nor PTZ in Ringer's solution had no significant effects on the Na permeability mechanism. The present results make it unlikely that the convulsive action of PTZ is related to changes in the electrical properties of excitable membranes as measured at nodes of Ranvier.

This work was supported by grants from the Swedish Medical Research Council (Project No. 14X-4253) and the Karolinska Institute.

REFERENCES

- ANDERSEN P, GJERSTAD L & LANGMOEN I A. 1978. A cortical epilepsy model *in vitro*. In: *Abnormal neuronal discharges* (ed. N. Chazotzakis & M. Bolzoni) pp. 79-86. Raven Press, New York.
- BERGMAN C, MOYNER, W & STÄMPFLI R. 1968. Sustained spontaneous activity of Ranvier nodes induced by the combined action of TEA and lack of calcium. *Pflüger Arch Ges Physiol* 302, 4-37.
- BRISMAN, T. 1980. Potential clamp analysis of membrane currents in rat myelinated nerve fibres. *J Physiol (Lond)* 298, 171-184.
- DAVID, R. J. WILSON W A & ESCUETA A V. 1974. Voltage clamp analysis of pentylenetetrazol effects on *Aplysia* neurons. *Brain Res* 67, 549-554.
- DINGLEDMINE, R. & GJERSTAD, L. 1980. Reduced excitability during epileptiform activity in the *in vitro* hippocampal slice. *J Physiol (Lond)* (in press).
- DODGE, F A & FRANKENHAEUSER, B. 1958. Membrane currents in isolated frog nerve fibres under voltage clamp conditions. *J Physiol (Lond)* 143, 76-90.
- GUTHRIE, M J & PRINCE, D A. 1972. Thalamo-cortical relay neurons: synchronous invasion of spikes from cortical epileptogenic focus. *Science* 176, 424-426.
- KLEE, M R, FABER, D S & HEISS W H. 1973. Strychnine and pentylenetetrazol-induced changes of excitability in *Aplysia* neurons. *Science* 179, 1133-1134.
- LOUVEL, J & PUMAIN R. 1978. Ectopic action potential generation: Picrotoxin action on pyramidal tract fibers. In: *Abnormal Neuronal Discharges* (ed. N. Chazotzakis & M. Bolzoni) pp. 63-73. Raven NY.
- MATSUMOTO H & AJMONI-MARSAN D. 1964. Cortical cellular phenomena in experimental epilepsy: interictal manifestation. *Exp Neurol* 9, 286-304.
- NOBELS J L & PRINCE, D A. 1977. Presynaptic origin of picrotoxin after discharges at mammalian nerve terminals. *Brain Res* 138, 59-74.
- NOBELS J L & PRINCE, D A. 1978. Development of focal seizures in cerebral cortex: role of axon terminal bursting. *J Neurophysiol* 41, 1267-1281.
- NOBELS, J C. & PRINCE, D A. 1978b. Excitability changes in thalamocortical relay neurons during syn-

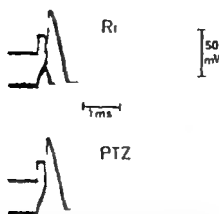


Fig. 1. Membrane action potentials (and subthreshold responses) in rat myelinated nerve fibre in Ringer's solution and test solution with 10 mM PTZ. Temp. 28°C.

brane potential. This method and the principles for calibration and analysis of the membrane currents have been described elsewhere (Brismar 1980). The potential clamp technique is similar to that developed by Dodge and Frankenhauser (1968) for the analysis of frog myelinated nerve fibres.

Test solutions with benzylpenicillin (Kabi) and pentylenetetrazol (Sigma) were prepared by adding these substances to the ordinary Ringer's solution (147 mM NaCl, 5.9 mM KCl, 3.0 mM CaCl₂, 5.0 mM Trizma® buffer of pH 7.4 at 25°C).

RESULTS

Membrane action potentials were recorded in Ringer's solution and after 5 min in 10 mM PTZ Ringer and no change was detected in the threshold, the amplitude and the shape of the action potential. There was neither any effect on the resting potential, which remained at the level to which it previously had been set by the balance control.

The membrane currents were recorded in potential clamp conditions. Step changes in membrane potential were made from resting level (U) to various values (V) and the consequent current was plotted against the amplitude of the step (Fig. 2). The membrane currents and the biophysical properties of normal rat myelinated fibres are described in Brismar (1980). In 10 mM PTZ (Fig. 2) and similarly 10 mM Pz had no effect on the curve of initial current vs. potential or the time course (τ_h) and steady state level of sodium inactivation.

The steady state currents associated with changes in potential are plotted in Fig. 3. In 10 mM PTZ or 10 mM Pz in Ringer's solution the amplitude of the steady state current was unchanged, indicating that the normal low permeability K⁺ current

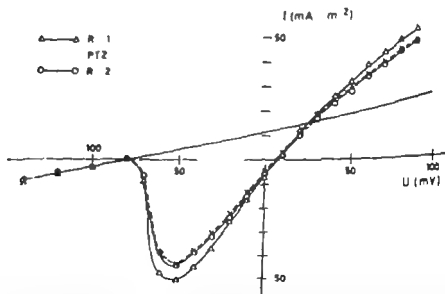


Fig. 2. Peak initial current vs. potential curves in Ringer's solution after 5 min in 10 mM PTZ Ringer and after wash out. Current amplitude measured after step change in potential to different values as indicated on abscissa. Holding potential -80 mV. Pz inactivation removed by 1 ms conditioning pulse to -120 mV. Smooth curves fitted to symbols. Leak current extrapolated from measured inward current at negative steps. Temp. 26°C.

Local cerebral glucose consumption during insulin induced hypoglycemia and in the recovery period following glucose administration

ABDUL RAHMAN and B. K. SIESJÖ

Laboratory of Experimental Brain Research and the Department of Neurosurgery, University of Lund, Sweden

ABDUL RAHMAN, A. & SIESJÖ, B. K. Local cerebral glucose consumption during insulin-induced hypoglycemia, and in the recovery period following glucose administration. *Acta Physiol Scand* 1980, 110: 149-159. Received 17 Jan 1980, ISSN 0001-6772. Laboratory of Experimental Brain Research and Department of Neurosurgery, University of Lund, Sweden.

Using the ^{14}C -deoxyglucose technique we estimated local glucose consumption in the rat brain ($\text{I-CMR}_{\text{glc}}$) in hypoglycemia of sufficient severity to cause cessation of spontaneous EEG activity and in the recovery period following 30 min period of such hypoglycemia. After 5 and 30 min of hypoglycemia, $\text{I-CMR}_{\text{glc}}$ was markedly reduced in many cerebral structures (cerebral cortex, cingulate gyrus, thalamus, and hippocampus) but unchanged or only moderately reduced in other structures (cerebellar cortex, hypothalamus, and pons). The results indicate that differences in $\text{I-CMR}_{\text{glc}}$ were caused by regional differences in the true or apparent kinetic constant for glucose transport, or that the consequences of hypoglycemic derangement of cellular metabolism interfered with glucose transport in some regions. Local CMR_{glc} was markedly heterogeneous in the recovery period induced by glucose administration in animals with a prior (30 min) period of hypoglycemia. In general, reduced posthypoglycemic glucose consumption was correlated to low $\text{I-CMR}_{\text{glc}}$ values during hypoglycemia. However, the hippocampus provided an exception since its CMR_{glc} returned to control values. A correlation with previous measurements of local cerebral blood flow (I-CBF) demonstrates that regions with pronounced reduction in $\text{I-CMR}_{\text{glc}}$ during hypoglycemia developed mismatch between blood flow and metabolic rate in the recovery period: the flow showing disproportional reduction.

Key words: Hypoglycemia, brain metabolism, local glucose consumption.

Measurements of cerebral blood flow (CBF) and of oxygen differences in glucose and oxygen not during hypoglycemia have demonstrated cerebral glucose consumption (CMR_{glc}) is reduced to a much larger extent than oxygen consumption (CMR_{O_2}) indicating that endogenous substrates are used to support energy production (Kety 1948, Enselberg & Seltzer 1962, Della Porta et al 1964, Gottstein & Held 1967, Pappenheimer & Bell 1973, Norberg & Siesjö 1976). When the hypoglycemia is severe enough to produce cessation of spontaneous electroencephalographic (EEG) activity and clinical coma CMR_{O_2} is, at least, only upheld at control levels (Della Porta et al 1964, Norberg & Siesjö 1976). However, energy production is nevertheless insufficient to cover the demands, and one observes marked perturbation of resting state with decreases in the tissue concentrations of phosphocreatine (PCr) and ATP and

increases in those of ADP and AMP (Hinzen & Möller 1971, Lewis et al 1974a, Norberg & Siesjö 1976).

Part of the endogenous substrate that is mobilized in hypoglycemia emanates from the tissue stores of glycolytic metabolites, citric acid cycle intermediates and free amino acids (Goldberg et al 1966, Lewis et al 1974b, Norberg & Siesjö 1976, Agardh et al 1978). However, evidence exists that severe hypoglycemia leads to consumption of structural components as well. Decreases in tissue concentrations of lipids and proteins were observed when isolated cat brains were perfused with glucose free blood (Aboud & Geiger 1955). In intact animals, decreases in phospholipid components have been noted (Hinzen et al 1970, Agardh et al in preparation). Presumably degradation of intracellular membranes contributes to the irreversible neuronal damage that occurs in severe pro-

- chronic discharges in cat neocortex. *J Neurophysiol* 41: 178-1796.
- PELLMAR T. C. & WILSON W. A. 1976 Penicillin effects on ionophoretic responses in *Aplysia californica*. *Brain Res* 116: 89-101.
- PELLMAR T. C. & WILSON W. A. 1977 Synaptic mechanism of pentylenetetrazole selectivity for chloride conductance. *Science* 197: 91-914.
- PRINCE D. A. & SCHWARTZKROIN P. A. 1978 Nonsynaptic mechanism in epileptogenesis. In *Abnormal neuronal discharges* (ed. N. Chazotte & M. Bousson) pp. 11. Raven N.Y.
- ROSEN A. D., VASTOLA E. F. & HILDEBRAND Z. J. 1971 Visual radiation activity during a cortical penicillin discharge. *Exp Neurol* 40: 1-11.
- SCHWARTZKROIN P. A., MUTANI R. P. & D. A. 1975 Orthodromic and antidromic cortical epileptic focus on ventral cat. *J Neurophysiol* 38: 794-811.
- SCOBEY R. P. & GABOR A. J. 1971 Epileptic potential generation in epileptogenic cortex. *J Physiol* 38: 381-394.
- VOSKUYL R. A. & ter MEURS H. E. D. 1978 Excitability increase of neurons in slices of the guinea pig after penicillin administration. *Brain Res* 146: 83-96.
- WILLIAMSON T. L. & CRILL W. E. 1973 Effects of pentylenetetrazol on masticatory neuronal activity: recording and stimulation. *Neurophysiology studies*. *Brain Res* 116: 17-29.
- WILSON W. A. & ESCUETA A. V. 1974 GABA synaptic effects of pentylenetetrazol and picrotoxin. *Brain Res* 77: 168-171.

Cerebral glucose consumption during insulin-induced hypoglycemia and in the recovery period following glucose administration

ABDUL RAHMAN and B. K. SIESJÖ

Department of Experimental Brain Research and the Department of Neurosurgery, University of Lund, Sweden

ABDUL RAHMAN A & SIESJÖ B K. Local cerebral glucose consumption during insulin-induced hypoglycemia, and in the recovery period following glucose administration. *Acta Physiol Scand* 1980 110: 149-159. Received 17 Jan 1980, ISSN 0001-6772. Laboratory of Experimental Brain Research and Department of Neurosurgery, University of Lund, Sweden.

Using the ^{14}C -deoxyglucose technique, estimated local glucose consumption in the rat brain ($\text{I-CMR}_{\text{glc}}$) in hypoglycemia of sufficient severity to cause cessation of spontaneous EEG activity and in the recovery period following a 30 min period of such hypoglycemia. After 5 and 30 min of hypoglycemia, $\text{I-CMR}_{\text{glc}}$ was markedly reduced in many cerebral structures (cerebral cortex, caudateputamen, thalamus, and hippocampus) but unchanged or only moderately reduced in other structures (cerebellar cortex, hypothalamus and pons/midbrain). The results indicate that differences in $\text{I-CMR}_{\text{glc}}$ were caused by regional differences in the true or apparent kinetic constant for glucose transport, or that the consequences of hypoglycemic derangement of cellular metabolism interfered with glucose transport in some regions. Local CMR_{glc} was markedly heterogeneous in the recovery period induced by glucose administration in animals with prior (30 min) period of hypoglycemia. In general, reduced posthypoglycemic glucose consumption was correlated to low $\text{I-CMR}_{\text{glc}}$ values during hypoglycemia. However, the hippocampus provided an exception since its CMR_{glc} returned to control values. A correlation with previous measurement of local cerebral blood flow (I-CBF) demonstrates that regions showing a pronounced reduction in $\text{I-CMR}_{\text{glc}}$ during hypoglycemia developed a mismatch between blood flow and metabolic rate in the recovery period, the flow showing disproportional reduction.

Key words: Hypoglycemia, brain metabolism, local glucose consumption.

measurements of cerebral blood flow (CBF) and of obvious differences in glucose and oxygen metabolism during hypoglycemia have demonstrated that cerebral glucose consumption (CMR_{glc}) is reduced to a much larger extent than oxygen consumption (CMR_{O_2}) indicating that endogenous substrates are used to support energy production (Kety 1948, Ehrenberg & Selinger 1962, Della Porta et al 1964, Gottstein & Heide 1967, Pappenheimer & Wolf 1973, Norberg & Siesjö 1976). When the hypoglycemia is severe enough to produce cessation of spontaneous electroencephalographic (EEG) activity and clinical coma CMR_{glc} is, at least in the rat, upheld at control level (Della Porta et al 1964, Norberg & Siesjö 1976). However energy

production is insufficient to cover the metabolic needs, and one observes marked perturbations of energy state with decreases in the tissue concentrations of phosphocreatine (PCr) and ATP and

increases in those of ADP and AMP (Hinzen & Möller 1971, Lewis et al 1974a, Norberg & Siesjö 1976). Part of the endogenous substrate that is mobilized in hypoglycemia emanates from the tissue stores of glycolytic metabolites, carboxylic acid cycle intermediates, and free amino acids (Goldberg et al 1966, Lewis et al 1974b, Norberg & Siesjö 1976, Agardh et al 1978). However, evidence exists that severe hypoglycemia leads to consumption of structural components as well. Decreases in tissue concentrations of lipids and proteins were observed when isolated rat brains were perfused with glucose-free blood (Aboud & Gelzer 1955). In intact animals, decreases in phospholipid components have been noted (Hinzen et al 1970, Agardh et al in preparation). Presumably degradation of intracellular membranes contributes to the irreversible neuronal damage that occurs in severe pro-

longed hypoglycemia (Meyer 1963; Brierley et al 1971; Agardh et al 1980).

Previous information on cerebral blood flow and metabolic rates in hypoglycemia pertains to the whole brain or to the cerebral cortex. Since neuronal damage after hypoglycemia shows preferential localization to certain regions (mainly cerebral cortex and hippocampus) it seems highly warranted to obtain information on regional blood flow and glucose consumption. Recently we described changes in local CBF during hypoglycemia and in the recovery period following glucose administration (Sjögaard & Abdul-Rahman 1979; Abdul-Rahman et al 1980). The results showed that during severe hypoglycemia CBF increased 2- to 3-fold in all structures visualized by the autoradiographic technique and that a pronounced hypoperfusion developed in many regions in the posthypoglycemic recovery period. The present study concerns local glucose consumption ($l\text{-CMR}_{gl}$) as measured with the autoradiographic ^{14}C -deoxyglucose technique of Sokoloff et al (1977). The study was designed to provide answers to the following questions: (1) Is there a uniform decrease in local $l\text{-CMR}_{gl}$ in the brain during moderate and severe hypoglycemia or are regional differences observed? This problem was approached by measurements of $l\text{-CMR}_{gl}$ in insulin treated rats that had either no EEG changes, an EEG pattern of slow waves/polyspikes or cessation of spontaneous EEG activity for 5 or 30 min. (2) Is local $l\text{-CMR}_{gl}$ during hypoglycemia determined by the glucose availability i.e. by blood flow and arterial glucose concentration or are additional mechanisms involved? In answering this question data for $l\text{-CMR}_{gl}$ were correlated to previous results on $l\text{-CBF}$. (3) Is there a posthypoglycemic depression of $l\text{-CMR}_{gl}$ corresponding to the reduction in local CBF? If this is so, is the depression of metabolic rate uniform or is it confined to certain regions? In the experiments performed to answer this question animals were given glucose after a 30 min period of hypoglycemic "coma" and $l\text{-CMR}_{gl}$ was measured 90 min later.

METHODS

1. Operative and sampling techniques

The experiments were performed on male Wistar rats, weighing 315-410 g. The animals were starved for 24 h but had free access to tap water. They were then injected with insulin (Novo Actrapid, Novo Industri AB) in a dose of

40 IU/kg i.p. After 60 min the animals were anesthetized with 5-3% halothane. When anaesthesia was tracheotomized, connected to a Starik respirator paralyzed with tubocurarine chloride (0.1 mg i.v.) and ventilated on 10% halothane and 3% Respiration was adjusted to give arterial PO_2 of 35-40 mmHg. Body temperature as rectum was kept close to 37°C by means of a heater.

Both femoral arteries were cannulated, one for pressure recordings and the other for sampling for analyses of pH, blood gases, glucose concentration and ^{14}C -activity. The latter catheter was cut to 1.5-2 cm to avoid smearing. One femoral vein was cannulated for infusion of ^{14}C -deoxyglucose and for infusion of glucose during the recovery period. Implanted screws were inserted through the skull bone to make contact with the dura and connected to an Elema EEG machine. In some animals a small hole was made over the superior sagittal sinus to allow sampling of cerebral venous blood in parallel with one end drawn out in a fine pipette. All operative procedures were completed, halothane supply continued and the animals were ventilated on 10% and 30% O_2 .

Experimental groups

Following the injection of insulin in a dose 40 IU/kg blood glucose concentrations were rapidly reduced. In the artificially ventilated rat, the hypoglycemia leads to typical EEG changes, such as an increased incidence of slow waves, proper slow wave polyspike pattern and ending in complete spontaneous electrical activity (Lewin et al 1974). In the present study the animals were grouped according to EEG pattern as follows:

(a) *Control*. The values used were those previously obtained in animals that were starved for 18-20 h (Sjögaard et al 1980).

(b) *Asymptomatic hypoglycemia*. In this group ($n=4$) insulin was injected after completing the procedure and ^{14}C -deoxyglucose was infused. Plasma glucose concentrations had decreased to about 0.5 mmol/l. At these concentrations the EEG was normal and remained so during the time of sampling.

(c) *Moderate hypoglycemia*. In these animals ($n=4$) EEG showed a markedly increased incidence of slow waves at the time of the ^{14}C -deoxyglucose-infusion. Plasma glucose concentrations were in the range 0.2-0.5 mmol/l.

(d) *Severe hypoglycemia*. All animals in this group showed a cessation of EEG activity for either 5 min ($n=3$) or 30 min ($n=4$) before infusion was begun. Plasma glucose concentrations ranged between 0.08 and 0.25 mmol/l.

Four separate animals were allowed a 20 min period of electroactivity before six consecutive samples of arterial and cerebral venous blood were simultaneously drawn every second min. After the last samples were drawn (30 min of electroactivity) ^{14}C iodantipyrine was given for 30 s, whereafter the animal was decapitated. The brain processed for the measurement of local CBF.

(e) *Recovery*. These animals ($n=4$) were allowed a 30 min period of "electroactivity" before recovery

Physiological parameters during insulin-induced hypoglycemia and in the recovery period following injection

means \pm S.E. Plasma glucose concentration is given in ranges. MABP = mean arterial blood pressure

	Temp (°C)	MABP (mmHg)	P ₁₀₀ (mmHg)	P ₁₀₀ (mmHg)	pH	Plasma glucose (μ mol ml ⁻¹)	EEG pattern
n=6	37.1 \pm 0.1	135 \pm 6	123 \pm 2	36.8 \pm 0.8	7.37 \pm 0.01	6.12-6.55	Normal
control asymptomatic	37.5 \pm 0.2	135 \pm 2	98 \pm 5	35.4 \pm 1.6	7.30 \pm 0.02	6.0 \pm 3.34	Normal
control Isoelectric (n=5)	37.0 \pm 0.2	115 \pm 5	108 \pm 4	34.4 \pm 1.2	7.32 \pm 0.03	0.6-1.26	Isoelectric
control Isoelectric (n=4)	36.9 \pm 0.2	108 \pm 1	114 \pm 6	33.8 \pm 1.7	7.36 \pm 0.04	0.58-1.10	Isoelectric
recovery 90'	36.8 \pm 0.2	113 \pm 3	99 \pm 4	36.6 \pm 1.1	7.35 \pm 0.06	9.1-13.74	Low voltage Slow waves

administration of glucose as an initial dose of 0.5 g/kg (w/v) solution, followed by constant infusion same solution at rate of 1 ml/kg for 90 min (et al 1978). At that time ¹⁴C-deoxyglucose was

value is yet available for plasma glucose concentrations of about 1 μ mol g⁻¹ i.e. values observed in animals with a ceased EEG activity. For these, a provisionally assumed lumped constant of 0.67 and performed separate experiments to control that I-CMR_{glc} values so obtained were reasonably close to those expected. However as the fol-

Measurement of local CMR and local CBF

CMR_{glc} was measured with the quantitative autoradiographic technique of Sokoloff et al. (1977). Details of the procedure have been given in previous communication from the laboratory (Bagyar et al. 1980). In 7 animals containing ¹⁴C-deoxyglucose (337 μ mol⁻¹ New England Nuclear) was infused i.v. over

The total dose injected was 25 μ Ci in hypoglycemic animals and 50 μ Ci in recovery animals. During the period of measurement, 14 timed 100 μ l arterial samples were taken (including bleed). The samples were strictly centrifuged and the plasma was separated and stored at -80°C for later determination of ¹⁴C-activity with scintillation counter. After the last (30 min) sample was decanted, the brain was removed and frozen in isopentane chilled to -50°C with nitrogen. Plasma glucose concentrations were determined with an enzymatic, fluorometric method based on hexokinase reaction, and ¹⁴C activity with liquid scintillation techniques. The ¹⁴C activity in the brain was determined by applying 20 μ sections to X-ray films with best densitometric measurements. By the use of these standards the tissue ¹⁴C activity could be calculated.

In calculation of I-CMR_{glc} according to Sokoloff et al. (1977) "lumped constant" is used which takes into account, among other things, the K_m and V_{max} values for transport of glucose and ¹⁴C-deoxyglucose between blood and brain. Under normal conditions the value of this is 0.67 (Sokoloff et al. 1977). In hypoglycemia, it shows an inverse relationship with plasma glucose concentration (Sokoloff et al., work in progress). Sokoloff was kind enough to provide us with provisional values for plasma glucose concentrations of about 0.67 and about 3 μ mol ml⁻¹ (0.69). No

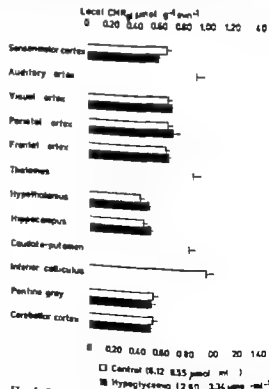


Fig. 1. Local cerebral glucose consumption (CMR_{glc}) in control animals and in animals with moderate insulin-induced hypoglycemia. The values are means \pm S.E. A significant change in CMR_{glc} was observed only in the caudate-putamen ($P < 0.05$).

Table 2 Local glucose consumption (I-CMR_g) in insulin-induced hypoglycemia

Structures	Control (n=6)	Isoelectric 5 (n=5)	Isoelectric 30' (n=4)
A. Superficial cerebral structures			
Cortex			
1 Sensorimotor	0.68±0.004	0.79±0.03	0.74±0.06
2 Auditory	0.97±0.07	0.11±0.04	0.40±0.07*
3 Visual	0.69±0.03	0.6±0.03	0.29±0.05
4 Parietal	0.69±0.04	0.79±0.03	0.34±0.06
5 Frontal	0.67±0.03	0.4±0.01	0.26±0.04
B. Deep cerebral structures			
6 Thalamus	0.89±0.07	0.43±0.04	0.35±0.06
7 Hypo thalamus	0.45±0.03	0.41±0.04	0.15±0.06
8 Hippocampus	0.47±0.03	0.20±0.02	0.3±0.04
9 Caudate-putamen	0.86±0.04	0.3±0.03	0.7±0.05
C. Mid brain and pons			
10 Inferior Colliculus	1.00±0.06	0.56±0.07*	0.56±0.07*
11 Pontine gray	0.55±0.04	0.4±0.04	0.37±0.05
D. Cerebellum			
1 Cerebellar cortex	0.55±0.03	0.40±0.06	0.4±0.09

The values are given in $\mu\text{mol/g} \cdot \text{mm}$ and represent means \pm S.E. * $P<0.05$ $P<0.01$ and $P<0.001$

following discussion will emphasize the I-CMR_g values obtained in hypoglycemia are only approximate and the major information of the study concerns differences in I-CMR_g between various brain structure (see Discussion).

Local CBF was measured autoradiographically with ^{14}C -iodoantipyrine as the diffusible tracer (Sakurada et al 1978 for details see Abdul Rahman et al 1979).

RESULTS

Table 1 shows values for physiological parameters measured at the time of ^{14}C -deoxyglucose infusion. All groups had mean body temperatures close to 37°C, arterial P_{O_2} values above 90 mmHg, P_{CO_2} values in the range 31–37 mmHg and pH values in the range 7.30–7.37. In the three most severely hypoglycemic groups and in the recovery group mean arterial blood pressure was reduced. However, no single animal had a blood pressure lower than 100 mmHg. The plasma glucose concentrations given (ranges) are the means of 14 values measured during the 30 min following ^{14}C -deoxyglucose administration. Also given in the table are the predominant EEG pattern observed during this period.

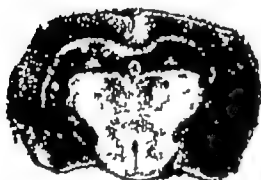
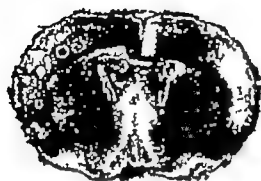
Regional changes in CMR_g during hypoglycemia

In one group of animals ^{14}C -deoxyglucose was infused when plasma glucose concentrations were

close to 3 $\mu\text{mol/g}$ (range 2.6–3.3 $\mu\text{mol/g}$). In the EEG showed an EEG pattern unaltered from control. In all animals plasma glucose concentrations remained within narrow limits during the 30 min period of blood sampling. Fig. 1 shows I-CMR_g values in a control group of six animals which did not receive insulin (lumped constant=0.48) to those seen in a plasma glucose concentration of 1 $\mu\text{mol/g}$ (lumped constant=0.69). Except for a slight increase in I-CMR_g in the caudoputamen, could well be fortuitous. The insulin-induced hypoglycemic animals had I-CMR_g values like those of the controls. We conclude that the onset of hypoglycemia which is moderate enough to leave the EEG pattern unaltered does not alter I-CMR_g.

In the moderately hypoglycemic animals with plasma glucose concentrations 1.7–2.7 $\mu\text{mol/g}$ the initial EEG pattern was one with slow waves. However, this functional state could not be explained. The EEG changed appreciably during the blood sampling period and in two animals EEGs were recorded. In view of this unsteady state situation, since the values obtained did not show variations in I-CMR_g, they will not be further considered.

Local CMR_g values obtained in isoelectric

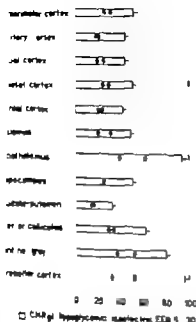


Local variations in cerebral glucose consumption are hypoglycemia (plasma glucose concentration 0.6 mM). The autoradiographs are processed in the autoradiography equipment of the Laboratory of Cerebral Metabolism, NIMH. Methods by courtesy of L. Sokoloff (Gosse, Arzoo & Sokoloff 1980). A provisional kinked constant of 0.87 was used the

colour scale is only semi-quantitative. The approximate CMR values are for red 0.30, yellow 0.20 and green 0.15 $\mu\text{mol/g min}$. The three sections given are representative of the following levels. Top: sensorimotor cortex, caudate-putamen and lateral striatum. Middle: Parietal cortex, thalamus, hypothalamus and hippocampus. Bottom: Cerebellar cortex.

Local C.R._{gl} as percent of control

0 20 40 60 80 100



Local cerebral glucose consumption in hypoglycemia of sufficient severity to cause cessation of EEG for either 5 or 30 min. The stippled denote pooled (5-30 min) for animals with plasma glucose concentrations in the range 1.03-1.26 $\mu\text{mol ml}^{-1}$ (mean \pm 7). The dots denote individual values measured (5 and 30 min) with plasma glucose concentrations of 0.58 and 0.60 $\mu\text{mol ml}^{-1}$ respectively. In all areas except the hypothalamus and the cerebellar C.R._{gl} in hypoglycemia was significantly reduced overall ($P < 0.05$).

were much more consistent) and the above values obtained were close to those expected from independent measurements (see below). In all animals except two plasma glucose concentrations at 0 and 30 min differed by less than 20%. Table 2 shows values obtained when ^{14}C -deoxyglucose was infused 5 min or 30 min after cessation of EEG activity. There were no systematic differences between values obtained in the 5 and 30 min groups. Values were therefore pooled for those experiments in which the mean plasma glucose concentrations fell within the narrow range of 1.26 $\mu\text{mol g}^{-1}$ ($n=7$). Excluded from this group were two experiments in which mean plasma glucose concentrations were 0.58 and 0.60 $\mu\text{mol g}^{-1}$. Local C.R._{gl} for these two ranges of mean glucose concentration are given in Fig. 2 (in

percent of control). The result of Table 1 and Fig.

demonstrate a marked reduction in 1-CMR_{gl} in cerebral cortical structures, thalamus, hippocampus and caudate-putamen, a less marked reduction in inferior colliculus and pontine grey and no reduction in hypothalamus or cerebellar cortex. The result also show a further pronounced reduction in 1-CMR_{gl} with a fall in plasma glucose from 1.1 to 0.6 $\mu\text{mol ml}^{-1}$.

The results described were obtained by taking the average of multiple densitometric readings from the same structure. This procedure and the large aperture of the densitometer hampered resolution of localized changes in 1-CMR_{gl} . The results of Fig. 3 which were obtained with the scanning densitometer of the Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda (Gooch et al. 1980) offer a finer resolution. The results emphasize the low CMR_{gl} in cerebral cortical structures, caudate-putamen, and hippocampus as well as the high CMR_{gl} in hypothalamus and cerebellar cortex. However they also show high glucose consumption values in structures not visualized by the manual densitometer (e.g. septal nuclei and plexus choroideus) and a marked heterogeneity in other structures, mainly the thalamus.

In order to study whether calculation of 1-CMR_{gl} with a lumped constant of 0.87 gives values close to the true ones, regional glucose consumption was calculated from arteriovenous differences in glucose content and 1-CBF , the latter measured autoradiographically with ^{14}C -iodoantipyrine. In the calculations shown in Table 3 we assumed that venous samples drawn from the superior sagittal sinus mainly reflect blood flow in the frontal and parietal cortex and we therefore derived CMR_{gl} for these structures. In one of the experiments two AVD_{gl} values were negative and the calculated mean value was close to zero. The results of the other three experiments suggested a direct relationship between plasma glucose concentration and AVD_{gl} as well as between plasma glucose concentration and CMR_{gl} (see below). In 2 expts. the plasma glucose concentrations were sufficiently similar to those of the two groups of Fig. 2 to allow a comparison of CMR_{gl} values. The CMR_{gl} values within parentheses are those measured with the deoxyglucose technique at plasma glucose concentrations of about 0.6 ($n=2$) and about 1.1 ($n=7$) $\mu\text{mol ml}^{-1}$. As the results show, there was a (a)

Table 3 Arteriovenous differences in glucose concentration (AVD_{gl}) cerebral blood flow (CBF) calculated cerebral glucose consumption (CMR_{gl}) related to plasma glucose concentration in hypoglycemia

Plasma glucose concentrations and AVD_{gl} values are means \pm S.E. ($n=6$). CMR_{gl} values given are from those measured autoradiographically at similar plasma glucose concentrations (see text)

Rat No	Plasma glucose ($\mu\text{mol ml}^{-1}$)	AVD_{gl} ($\mu\text{mol ml}^{-1}$)	CBF $\text{ml g}^{-1} \text{min}^{-1}$		CMR_{gl} $\mu\text{mol g}^{-1} \text{min}^{-1}$	
			Frontal	Parietal	Frontal	Parietal
1	0.34 ± 0.07	0.04 ± 0.01	14	69	0.09	0.11
	0.41 ± 0.04	0.01 ± 0.01	1.87	74	0.02	0.02
3	0.67 ± 0.10	0.06 ± 0.0	97	3.4	0.18 (0.15)	0.21 (0.15)
4	0.91 ± 0.11	0.10 ± 0.01	3.94	5.0	0.39 (0.30)	0.50 (0.40)

agreement between CMR_{gl} calculated from CBF and AVD_{gl} and those measured with the deoxy glucose technique

Local CMR_{gl} and glucose availability

The present results demonstrate that within any given structure CMR_{gl} was tightly correlated to plasma glucose concentration. This is exemplified for frontal cortex and the hypothalamus in Fig. 4. As already stated a similar relationship was obtained when CMR_{gl} was calculated from AVD_{gl} and CBF (see Table 3).

The dependence of glucose consumption on plasma glucose concentration does not explain the

regional differences in CMR_{gl} . Possibly differences could reflect corresponding differences in glucose availability i.e. in the product of arterial glucose concentration and CBF. Our measurements of AVD_{gl} (see Table 3) indicate that CBF is not an important determinant of glucose availability (and hence of CMR_{gl}) (Lund Andersen 1979). Thus, since AVD_{gl} did not exceed 10% of plasma concentration, we suggest that the arterial plasma glucose concentration is the dominant factor determining glucose availability. We conclude that although the plasma glucose concentration determines CMR_{gl} in any given structure differences between structures are caused by other factors (see Discussion).

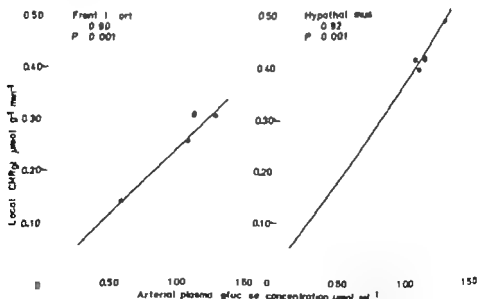


Fig. 4 Individual values for glucose consumption (CMR_{gl}) in frontal cortex and hypothalamus during severe hypoglycemia related to plasma glucose concentrations. The values relate to a period of hypoglycemic coma of either 5 (open circles) or 30 (filled circles) min duration.

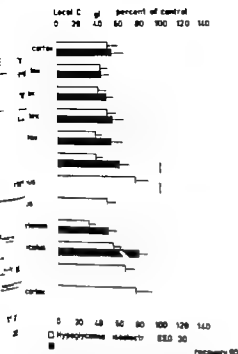


Fig. 5. Local cerebral glucose consumption (CMR_{gl}) in every period following 30 min period of severe hypoglycemia, as related to CMR_{gl} at the end of the recovery. The values are means \pm S.E. in percent of control. During recovery CMR_{gl} was reduced below control (100) in all structures except the hypothalamus, pons, medulla and cerebellar cortex.

CMR_{gl} in the recovery period

CMR_{gl} in the posthypoglycemic recovery as measured 90 min after the start of administration, following 30 min period of hypoglycemia with ceased EEG activity. In Fig. 5 the values for CMR_{gl} have been compared to measured in the 30 min hypoglycemic group. CMR_{gl} was markedly heterogeneous in the recovery period. In many structures (cerebral cortex and caudate-putamen) the values reduced to 45–60% of control while in others (hypothalamus, hippocampus, inferior colliculus, pons, medulla and cerebellar cortex) values were close to control. In general areas with markedly reduced CMR_{gl} during hypoglycemia showed low values in the recovery period, and those having moderately reduced CMR_{gl} during hypoglycemia showed recovery values close to control. However one notable exception was the hippocampus whose glucose consumption rate related to control values in the recovery period.



Fig. 6. A comparison between local cerebral glucose consumption (CMR_{gl}) and blood flow (CBF) after 90 min of recovery following 30 min period of severe hypoglycemia. The values are means \pm S.E. in percent of control values. The CBF values were taken from Abdul-Rahman et al. (1980).

The results presented raise the question whether or not the previously reported reduction in 1-CBF during the recovery period (Abdul-Rahman et al. 1980) merely reflects a reduced metabolic rate. In order to evaluate this possibility local glucose consumption rates were arranged in the order of increasing values and compared to the corresponding 1-CBF values. As Fig. 6 shows 1-CBF was reduced out of proportion to the reduction in 1- CMR_{gl} in all cortical areas and in the hippocampus there was pronounced mismatch between metabolic rate and blood flow.

DISCUSSION

The present results give novel information on local cerebral glucose consumption in severe hypoglycemia and they provide answers to the questions raised in the introduction. Before these results are discussed it seems warranted to briefly consider the validity of the deoxyglucose technique when applied to severe hypoglycemia.

The deoxyglucose technique in hypoglycemia

The unique feature of the ^3C -deoxyglucose technique of Sokoloff et al. (1977) is its ability to measure local glucose consumption. In hypoglycemia glucose consumption is no longer synonymous with metabolic rate since endogenous substrates partly substitute for the exogenous glucose. Thus the technique then estimates glucose delivery to structures in the brain rather than their metabolic rates. In all probability, glucose delivery is inversely proportional to the derangement of local cerebral metabolism caused by the hypoglycemia. Information on glucose delivery during hypoglycemia and on posthypoglycemic metabolic rates seems a prerequisite for understanding the preferential localization of neuronal lesions to certain brain structures.

One problem of applying the technique to hypoglycemia is that the lumped constant varies with plasma concentration (Sokoloff et al. in preparation). Since the main emphasis in the present work was on regional differences in CMR_{glc} we chose to use the provisional lumped constants obtained by Sokoloff et al.

In the isoelectric groups values for CMR_{glc} were reproducible, presumably because plasma glucose concentrations remained within narrow limits. Two findings indicate that the absolute CMR_{glc} values that were obtained with a lumped constant of 0.87 were reasonably accurate. First, at this degree of hypoglycemia previous calculations of CMR_{glc} from CBF and AVD_{glc} indicate a reduction of glucose consumption to about 40% of control (Norberg & Siesjö 1976). Second, similar calculations performed in the present study gave CMR_{glc} values approximating those obtained with the ^3C -deoxyglucose technique (Table 3).

Since the deoxyglucose technique gives CMR_{glc} values for a 30 min steady state period it may be asked if values obtained in the present 5 and 30 min groups really pertain to these periods of hypoglycemia "coma". Two facts indicate that the values obtained are good approximations of "early" and "late" hypoglycemic coma. First, CMR_{glc} values normally obtained are heavily weighted in favour of the first 15 min following deoxyglucose infusion (Sokoloff 1978). Second, since severe hypoglycemia reduces tissue glucose concentrations to vanishingly small values the precursor pool must show a very rapid turnover.

Regional differences in CMR_{glc} during hypoglycemia

The present results clearly demonstrate differences in CMR_{glc} occur in severe hypoglycemia. For example, in the 5 and 30 min groups structures that normally have a low CMR_{glc} (e.g. cerebellar cortex and pons) give CMR_{glc} values greatly exceeding those of other areas (e.g. cerebral cortex). Two questions must be posed. First, does the close relation between plasma glucose concentration and CMR_{glc} reflect a rate-limiting transport of glucose from blood to tissue? Second, if this is so, what are the local differences in CMR_{glc} ? In order to answer these problems we must consider glucose transport between blood and tissue.

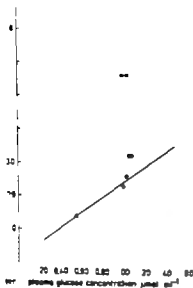
Current information on glucose flux between blood and brain suggests that it occurs by a transport via a symmetrical mobile carrier, the rate-limiting step being mainly located at the blood-brain barrier (Lund-Andersen 1976; Siesjö 1978). If this information is correct, transport can be described by the equation

$$\text{Net flux} = \frac{T_{\text{max}} \cdot C}{C + K} - \frac{T_{\text{max}} \cdot C_2}{C_2 + K_1}$$

where C and C_2 are the concentrations of glucose on either side of the blood-brain barrier, T_{max} is the maximal transport rate, and K is the affinity constant between glucose and transport mechanism. In severe hypoglycemia C_2 can be set to zero (Siesjö et al. 1974a). Assuming a K of $2 \mu\text{mol/l}$ and a T_{max} of $2.2 \mu\text{mol/g} \cdot \text{min}^{-1}$ (see Borjesson et al. 1976) we can provisionally calculate net flux for plasma glucose concentration range $1-10 \mu\text{mol/ml}$ and compare the values obtained with those measured with the deoxyglucose technique in the 5 and 30 min groups. In Fig. 7 data are shown for the frontal cortex and inferior colliculus. The results demonstrate that $1-\text{CMR}_{\text{glc}}$ in frontal cortex, which exemplifies the conditions in areas with low CMR_{glc} , closely reflects influx of glucose from plasma. However, in colliculus inferior a relative high- CMR_{glc} structure glucose consumption greatly exceeds the expected influx. Possible explanations are conveniently discussed on the basis of two alternative hypotheses.

(a) *The true kinetic constants show regional differences.* Clearly, the results obtained can be explained by regional variations in K and

Frontal cortex
inferior olivary



local cerebral glucose consumption (CMR_{glc}) in cortex and inferior olivary in severe hypoglycemia related to arterial plasma glucose concentration. The line represents the expected influx of glucose transport V_{max} of 22 $\mu\text{mol g}^{-1} \text{min}^{-1}$ and K_m of 20 $\mu\text{mol dl}^{-1}$.

For example if 'high CMR_{glc} ' areas have T_{max} or lower K_m values than those previously used they would tolerate a more severe hypoglycemia than other structures. In the fact that regional differences in glucose may determine the neuropathological outcome of severe hypoglycemia it seems highly warranted to obtain information on regional values for T_{max} .

The true kinetic constants are uniform throughout the brain. Obviously if this were the case T_{max} must be higher or K_m lower than the values must fit the areas with the highest CMR_{glc} values. Furthermore some factors have disrupted glucose influx in areas with high CMR_{glc} values. Two possibilities could be excluded. Firstly it is conceivable that the glucose transport could be inhibited by products of the metabolism e.g. free fatty acids (cf Hansson 1971). Secondly if cell edema compromises erythrocyte circulation e.g. by causing preferential flow of blood the effective T_{max} would be reduced.

Presently there is not sufficient information available

to distinguish between the possibilities considered and further work is required to explore the mechanisms underlying the pronounced regional differences in CMR_{glc} .

Regional differences in CMR_{glc} in the recovery period

The present results unequivocally demonstrate that a normal CMR_{glc} in the recovery period was observed only in a few cerebral structures while CMR_{glc} was severely depressed in others. It is tempting to conclude that areas with a low metabolic rate (e.g. cerebral cortical structures) suffered functional damage during hypoglycemia preventing a return of metabolic rate following glucose administration. This conclusion receives support from results on oxygen consumption which is also depressed in the recovery period (Agarwal et al. in preparation) as well as from neuropathological studies which demonstrate relatively extensive neuronal damage in the cerebral cortex (Agarwal et al. 1980; Kallamo et al. 1980). However the present results on the hippocampus demonstrate that a marked reduction in CMR_{glc} during hypoglycemia does not necessarily prevent a return of metabolic rate to control values.

The correlation between $1-CMR_{glc}$ and $1-CBF$ in the recovery period provides an interesting insight into events of probable importance in the pathophysiology of hypoglycemic cell damage. Those areas which are considered sensitive to hypoglycemic cell damage showed a mismatch between CMR_{glc} and CBF, the latter being reduced out of proportion to the depression of metabolic rate. This was especially evident in the hippocampus whose blood flow fell to 77% of control while CMR_{glc} returned to control. However since CBF was reduced to 40% of control after 30 min of recovery and to 40% after 180 min (Abdul-Rahman et al. 1980) the difference in results between the hippocampus and other regions (cerebral cortex and caudate-putamen) is less pronounced than what is indicated by Fig. 5.

Relation to neuropathology

Carefully controlled studies of the neuropathological alterations following hypoglycemia have previously only been carried out in monkeys (Brierley et al. 1971). In these insulin-induced hypoglycemia was sustained so as to abolish somatosensory-evoked potential for 49–97 min.

Neuronal alterations occurred diffusely in the neocortex and in some animals also in the hippocampus and caudate-putamen. Recently extensive studies on rats have been carried out with both light and electron microscopic techniques (Agardh et al 1980; Kalimo et al 1980). The results showed that a 30 min period of hypoglycemia with ceased EEG activity was accompanied by relatively extensive neuronal alterations in the cerebral cortex. So far, however, the studies have not been extended to include other parts of the brain. However, provided that changes are similar in the monkey and the rat, the present results, and those of our previous study (Abdul Rahman et al 1980), indicate that nerve cell damage occurs in areas that suffer a pronounced reduction in CMR_g during hypoglycemia and which show a marked mismatch between flow and metabolic rate in the recovery period.

This study was supported by grants from the Swedish Medical Research Council (project No. 14X-263) from US PHS (grant No. 5 RO1 NS07838) and from Knut and Alice Wallenberg's Foundation. We are grateful to Dr Sokoloff, Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, USA, for allowing us to use the lumped constant for hypoglycemia. The excellent technical assistance of Kerstin Beirup, Gunilla Öklid, Maj-Lis Smith, Karin Hansson, Barbro Asplund and Gerlie Johansson is gratefully acknowledged.

REFERENCES

- ABDUL RAHMAN A, DAHLOREN N, INGVAR M, REHNCRONA S & SIESJÖ B K 1979 Local versus regional cerebral blood flow in the rat at high (hypoxia) and low (phenobarbital anesthesia) flow rates. *Acta Physiol Scand* 106, 53-60.
- ABDUL RAHMAN A, AGARDH C D & SIESJÖ B K 1980 Local cerebral blood flow in the rat during severe hypoglycemia and the recovery period following glucose injection. *Acta Physiol Scand* 109, 307-314.
- ABOOD L O & GEIGER A 1955 Breakdown of proteins and lipids during glucose-free perfusion of the cat's brain. *Amer J Physiol* 182, 557-560.
- AGARDH C D, FOLBERGROVÁ J & SIESJÖ B K 1978 Cerebral metabolic changes in profound insulin-induced hypoglycemia and in the recovery period following glucose administration. *J Neurochem* 31, 1135-1142.
- AGARDH C D, KALIMO H, OLSSON Y & SIESJÖ B K 1980 Hypoglycemic brain injury: Metabolic and light microscopic findings in rat cerebral cortex during profound insulin-induced hypoglycemia and in the recovery period following glucose administration. *Acta Neuropath (Berl)*, 50, 31-41.
- BOROSTRÖM L, NORBERG K & SIESJÖ B K 1976 Glucose consumption in rat cerebral cortex during normoxia, hypoxia and hypercapnia. *Scand J Clin Lab Invest* 36, 569-579.
- BRIERLEY J B, BROWN A W & MELDORF J 1971 The nature and time course of the alterations resulting from oligosaccharide and hypoxia in the brain of *Macaca Mulatta*. *Brain Res* 44, 1-10.
- DELLA PORTA P, MAIOLO A T, AGRICOLA R, ROSSELLA, E 1964 Cerebral blood flow and metabolism in therapeutic insulin coma. *Neurology* 14, 131-140.
- EISENBERG S & SELTZER H S 1962 The metabolic effects of acutely induced hypoglycemia in human subjects. *Metabolism* 11, 110-118.
- GOLDBERG N D, PASSONNEAU J V & O H 1966 Effects of changes in brain metabolism on the levels of citric acid cycle intermediates. *Biochem J* 99, 399-403.
- GOOCHER C, RASBAND W R & SOKOLOFF L 1980 Computerized densitometry and calibration of ¹⁴C-deoxyglucose autoradiography. *Ann Neurol* 1, 110-118.
- GOTTSTEIN U & HELD K 1967 Insulin bei gesunden und Diabetikern. *Klin Wochenschr* 45, 1-10.
- HASSELBLATT A 1971 Interrelations between carbohydrate metabolism, Na⁺ and K⁺ metabolism. *Arch Pharmacol* 269, 313-346.
- HINZEN D H & MÜLLER U 1971 Einwechsel und Funktion des Kaninchengehirns bei Insulinhypoglykämie. *Pflügers Arch Ges Physiol* 47, 59.
- HINZEN D H, BECKER P & MÜLLER U 1972 Einfluss von Insulin auf den regionalen Stoffwechsel des Kaninchengehirns in vivo. *Pflügers Arch Ges Physiol* 231, 1-14.
- INGVAR M, ABDUL RAHMAN A & SIESJÖ B K 1980 Local cerebral glucose consumption in artificially ventilated rat: influence of anaesthesia and of phenobarbital anaesthesia. *Physiol Scand* 109, 177-185.
- KALIMO H, AGARDH C D, OLSSON Y & SIESJÖ B K 1980 Hypoglycemic brain injury: I. microscopic findings in rat cerebral cortex during profound insulin-induced hypoglycemia and the recovery period following glucose administration. *Acta Neuropath (Berl)* 50, 43-52.
- KETY S S, WOODFORD R B, HARVEY P, FREYHAN F A, APPEL K E & SCHWARTZ R 1948 Cerebral blood flow and metabolism in schizophrenia: The effects of barbiturate or costly insulin coma and electroshock. *Amer J Physiol* 154, 765-770.
- LEWIS L D, LJUNGGREN B, RATCHESON A & SIESJÖ B K 1974a Cerebral energy metabolism during hypoglycemia, related to blood flow and to EEG. *J Neurochem* 23, 673-679.
- LEWIS L D, LJUNGGREN B, RATCHESON A & SIESJÖ B K 1974b Changes in cerebral amino acid and amino acid metabolism during hypoglycemia and recovery. *Neurology* 24, 671-679.

- ANDERSEN H. 1979 Transport of glucose from blood to brain. *Physiol Reviews* 59: 305-332.
- ERQ, K. & SIESJÖ B. K. 1963 Intoxications. IN Greenfield Neurobiology (ed. W. Blackwood, W. H. McMenemey, J. P. Meyer, R. M. Naranjo & D. S. Russell), pp. 235-244. Williams & Wilkins, Baltimore.
- ERQ, K. & SIESJÖ B. K. 1976. Oxidative metabolism of the cerebral cortex of the rat in severe insulin-induced hypoglycemia. *J Neurochem* 26: 345-352.
- ENHEIMER, J. R. & SETCHELL, B. P. 1973 Cerebral glucose transport and oxygen consumption in sheep and rabbits. *J Physiol* 223: 529-551.
- ERQ, K., KENNEDY C., JEHL, J., BROWN D., CARBIN H. L. & SOKOLOFF L. 1978 Measurement of local cerebral blood flow with ^{14}C iontophoresis. *Amer J Physiol* 234(1): H159-H166.
- SIESJÖ B. K. 1978 Brain energy metabolism. John Wiley & Sons, Chichester, New York.
- SIESJÖ B. K. & ABDUL-RAHMAN A. 1979 Delayed hypoperfusion in the cerebral cortex of the rat in the recovery period following severe hypoglycemia. *Acta Physiol Scand* 106: 375-476.
- SOKOLOFF L. 1978 Local cerebral energy metabolism: its relationships to local functional activity and blood flow in Cerebral vascular smooth muscle and in control. *Ciba Foundation Symposium* 46 (ed. H. Purves), pp. 171-197. Excerpta Medica, Amsterdam, Oxford, New York.
- SOKOLOFF L., REIVICH M., KENNEDY C., DES ROSIERS M. H., PATLAK C. S., PETTIGREW K. D., SAKURADA O. & SHINOHARA M. 1977 The ^{14}C -deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure and normal values in the conscious and anaesthetized albino rat. *J Neurochem* 28: 897-916.

Output of bile and pancreatic enzymes after test meals with different fat content. Influence of body weight on pancreatic enzyme composition

LUND and C. JOHANSSON

Dept of Medicine, G-I Unit, Karolinska Hospital, S-141 86, Stockholm, Sweden

ERIKSSON, K. & JOHANSSON, C. Output of bile and pancreatic enzymes after test meals with different fat content. Influence of body weight on pancreatic enzyme composition. *Acta Physiol Scand* 1980, 110: 161-165. Received 17 Jan. 1980. ISSN 0001-6772. Department of Medicine, G-I Unit, Karolinska Hospital, Stockholm, Sweden.

The output of bile and pancreatic enzymes after two mixed meals with different fat content was studied in 16 healthy volunteers of normal body weight. A quantitative multiple indicator dilution method was used. The delivery of more fat per time to the duodenum after the fat rich compared to the low fat meal was followed by the emptying of more bile but did not significantly increase the pancreatic response. The ratio between amylase and lipase was constant throughout the course of single experiment but differed between the subjects. The ratio decreased with an increasing body weight, indicating a relative dominance of amylase over lipase in the lighter and the reverse in the heavy subjects. The same correlation was found when results from a previously reported series with a glucose containing mixed meal were reexamined. It is proposed that long time dietary differences might influence the enzyme composition of the postprandial pancreatic secretion.

Key words: Bile acid excretion, gastric emptying, indicator dilution technique, pancreatic secretion, test meal.

of the composition of a mixed meal after delivery of nutrients to the duodenum and thus stimulation to intestinal functions (Lagerstedt & Ekstrand 1976). In a previous study it is shown that the addition of more protein to a meal increases the gastric emptying rate of a meal and the secretion rate of pancreatic enzymes whereas the gallbladder emptying is unaffected (Ekstrand & Johansson 1975). This study reports quantitative measurements of the biliary and pancreatic response to mixed liquid meals with different fat content in healthy non-obese subjects.

with 3 polyvinyl tubes of different lengths. The set of 3 tubes is swallowed in the afternoon before the experimental day and are allowed to pass down the gastrointestinal tract by normal peristalsis. After an overnight fast the position of the tubes is checked by fluoroscopy. The tube tips should be positioned in the gastric antrum, in the duodenum descendens and in the jejunum 75 cm from the aortal tube. The gastric residual is removed before starting the experiment and salivary secretion is continuously withdrawn.

From the time of meal intake and on, the intestinal

Table 1 Composition of 1st meal (g/100 ml)

	NO low fat meal	NOF high fat meal
Skim milk protein	3.4	3.6
Lactose	5.1	5.1
Corn oil	3.9	11.8
Polyethylene glycol (PEG)	3.0	3.0
Number of subjects (females)	9 (2)	7 (2)
Body weight (M ± S.D.)	75 ± 13	74 ± 14

METHODS

Two 300 ml test meals differing in their content were administered to 16 normal healthy volunteers, 8 men and 8 women aged 20 to 38 years (Table 1), and compared, as obtained from each subject and the test was approved by the Ethical Committee at the Karolinska Hospital.

Experimental procedure. The multiple indicator dilution technique requires occlusion of the gastrointestinal tract

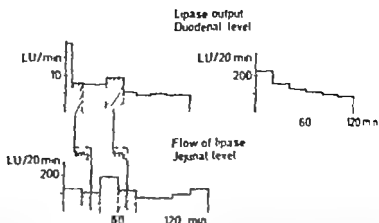


Fig. 1. Time relationship between the amount of lipase passing the jejunal sampling site and the rate of lipase output at the duodenal level expressed as LU/min or LU/20 min to facilitate comparison between different experiments as LU/20 min.

content are continuously marked with 3 different indicators infused through the duodenal tube at a constant rate (0.5 ml/min). The indicators, unlabelled vitamin B₁₂, ¹⁴Co-B₁₂ and ³H-Co-B₁₂ are sequentially infused with change of indicator every 40th minute.

Gastric samples are withdrawn at 20 min intervals. The jejunal contents are continuously siphoned by 20 min collections, and are kept at -20° until analysed.

Chemical method. Polyethylene glycol was determined according to Hyden (1945). Protein was determined with Reid's modification of the method by Folin-Ciocalteu (1959) and fat by titration of free fatty acids after complete hydrolysis. Carbohydrates were determined by the Hagedorn method (1935). Bilirubin was measured according to Jendryak & Groof (1938). Lipase according to Desnuelle, Constantin & Bakly (1955) and amylase according to Lagerlöf's modification of Nöbby's method (1935).

Calculation. A detailed description of the calculations has been given elsewhere (Lagerlöf et al. 1976). The duodenal indicators mix with the emptied gastric marker (PEG) during transit of the segment. The concentration of the mixed indicators in the jejunal collections are used to calculate flow rates of chyme at the jejunal sampling site (ml/20 min). By adjusting the scale of each indicator to the same magnitude the sequential infusions of 3 different duodenal indicators can be regarded as the infusion of one marker at a constant rate. The difference between the rates at which the indicator is infused into the duodenum and passes the exit of the test segment equals the change of transit time and the addition of consecutive changes expresses the varying transit times in the segment. The transit time is used to compensate for the time lag between the passage of test meal marker, pancreatic enzymes and bile at the jejunal level and their entrance into the duodenum. The output of bile and pancreatic enzymes are thereby adapted to the level of the Vater's sphincter (Fig. 1) and the gastric emptying of marker and content can be assessed. The emptying of the different meal components have to be calculated separately due to intragastric separation. The calculations are considered correct if the emptied amount equals the amount in the test meal.

Statistical methods. Results are given as the standard error of the mean (M ± S.E.). Samples were tested by the Wilcoxon test for unpaired samples and by the Spearman rank test.

Linear regressions were calculated between the amount of amylase to lipase with time.

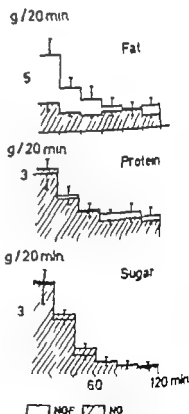


Fig. 2. The gastric emptying of different food components (M ± S.E.M. g/20 min) after the high fat meal (HF) and the low fat meal (NOF).

Correlation between body weight and gastric emptying during the first hour after intake of fat meal

Body weight kg	Gastric emptying	
	Energy (kJ 0-60 min)	Fat (g 0-60 min)
62	478	8.7
81	461	8.7
65	704	14.9
71	577	11.1
85	1341	17.2
87	985	26.5
100	40.05	40.05

TS

emptying. More fat was emptied to the colon during the first 40 min after the high fat meal than after the low fat meal (11 ± 1.7 g vs. 7 g, $P < 0.01$). The emptying of protein and carbohydrate was not affected by the added fat (Fig. 2). A positive correlation was previously reported between body weight and the early emptying of caloric energy from the low fat meal (Johansson 1974). A similar correlation was found also after the high fat meal (Table 2).

and loads to the duodenum did not differ between the meals, an average 22.6 ± 4.0 and 1.2 mmol/l duodenal acid being emptied after low and high fat meals respectively.

output of bile. After an initial gallbladder emptying, output of bile is approximately constant (10 mmol/h) and on (Fig. 3). More bile was emptied during the first 40 min after the fat rich meal compared with the meal with a lower content of fat (0.5).

output of pancreatic enzymes. The output rates of amylase and lipase were not significantly affected by the added fat (Fig. 3) and the total pancreatic response was not significantly different between the meals.

parallel secretion of the pancreatic enzymes. About the course of each experiment is indicated by the absence of any correlation between the amylase to lipase and time. The average b-values were -0.0029 and -0.0063 for the low and high fat meals respectively.

correlation was found between the body weight

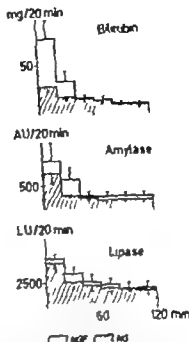


Fig. 3. The output of bile (mean \pm S.E.M. mg/20 min) and amylase (mean \pm S.E.M. AU/20 min) and lipase (mean \pm S.E.M. LU/20 min) at the level of papilla Vateri after the high fat meal (HGF) and the low fat meal (LGF).

and the pancreatic enzyme composition. The more heavily the subject the lower was the ratio between amylase and lipase (Table 3). In the high fat series the total output of amylase decreased at an increasing body weight.

DISCUSSION

The gastric emptying of liquid nonhyperosmotic meals is rapid in the beginning, then slowing to become almost constant (Johansson 1974). To this corresponds an initial rapid intestinal transit of the meal followed by a slower oscillatory intestinal propagation (Johansson 1974; Lagerlöf et al. 1976). Knowledge of the variations in transit times is prerequisite to adjust the jejunal flow of bile and enzymes to the output rate at the level of the Vateric sphincter. The enzyme output at this level follows the pattern of gastric emptying. The distribution of enzymes then changes along their transit along an intestinal segment and the postprandial enzyme profile is thus different intestinal levels. This may be one reason why, besides differences in duodenal stimulation

Table 3 Correlation between body weight and the total output of lipase and amylase after the meal

Subjects 1 and 4 were females

Exp No	Body weight (kg)	Broca's index x	Amylase (AU 10 ³)	Lipase (LU 10 ³)	AU/LU
1	62	0.86	7.5	12.3	0.61
2	64	0.85	1.7	12.7	0.14
3	65	0.87	5.5	13.8	0.23
4	71	1.01	2.4	9.3	0.26
5	85	1.08	0	22.7	0.09
6	87	0.97	1.3	13.4	0.10
7	100	1.04	0.7	15.0	0.05
r			<0.05	>0.05	<0.05

$$\text{Broca's index body} = \frac{\text{body weight}}{\text{length} - 100}$$

explains the different secretory patterns of pancreatic enzymes reported in literature (Brunner et al 1974 Ekelund & Johansson 1975 Hong Chin & Hur 1961). The delivery of more fat per time unit to the duodenum after the fat rich meal was followed by the emptying of more bile whereas the pancreatic enzyme response was not significantly increased. This supports the suggestion of a lower maximal threshold for pancreatic secretion than for gallbladder contraction (Brunner et al 1974).

The parallel secretion of the different enzymes during the course of each single meal is in agreement with the results of Hong et al (1961). Not reported before is the relation between body weight and the enzyme composition of the pancreatic secretion. The ratio between amylase and lipase was found to decrease at an increasing body weight due to a lowering of the amylase content. Differences in enzyme inactivation due to different transit times cannot explain these results. Heavy subjects do have shorter transit times than lighter (Johansson & Ekelund 1976) but variations are less marked between the subjects than during the course of a meal. In one subject and the ratio amylase to lipase was constant throughout each experiment. The intestinal loads of hydrochloric acid were not different in heavy and light subjects.

The present data are however insufficient to exclude the possibility that the early emptying of more fat and energy in heavy subjects rather than the body weight determines the pancreatic enzyme composition. Previously reported experiments with a hypertonic mixed meal (Lagerlöf et al 1976) were

therefore reexamined. This meal contained instead of fat as the dominant energy. Early emptying is unrelated to the body weight. Also after this meal the ratio between amylase and lipase was negatively correlated to the body weight (Fig. 4) and there existed a positive correlation between the body weight and the total lipase ($r=0.6217$ $P<0.02$, $n=15$).

The interrelationship between the body weight and the pancreatic enzyme composition can be interpreted in terms of a long time adaptation of the pancreatic gland to differences in food intake. The higher the caloric density of a meal the larger amount of energy delivered to the duodenum after meal intake as demonstrated from

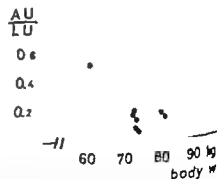


Fig. 4 The bodyweight is negatively correlated to the ratio between the total amount of amylase and lipase (AU/LU) ($r=-0.6863$ $P<0.01$ in 15 healthy subjects). The bodyweight (two females) after a 300 ml meal had the same composition as the low fat meal for the addition of 10 g glucose per 100 ml.

- by Hunt & Stubbs (1975) and own studies from 1976, unpublished data). Hunt & Cash also further found that heavy people prefer the higher energy density than to those of or less bodyweight. Different dietary habits and light subjects should thus imply different rates of stimulation of the pancreatic gland at periods of time. Evidence for long term influence on the enzyme composition has not been presented by Dreiling *et al.* (1962) and a low amylase content in pancreatic juice from obese patients, which was almost doubled 6 months of dietary restriction.
- Further studies have to be made to define causal relations between body weight, dietary habits and pancreatic enzyme composition. A direct consequence of the body weight-related variations in the enzyme composition is that clinical studies of the exocrine pancreatic function must include determinations of at least two
- DESNUELLE, P., CONSTANTIN, M. J. & BALDY, J. 1955. Mesure de l'activité de la Lipase. *Soc. Chén. Biol.* 37: 285-290.
- DREILING, D., ELSBACH, P., SCHIAFFNER, F. & SCHWARTZ, J. 1962. The effect of restriction of protein and total calories on pancreatic function in obese patients. *Gastrointest.* 42: 646-649.
- EKELUND, K. & JOHANSSON, C. 1975. Secretion of bile and pancreatic enzymes in response to meals of various compositions. *Scand. J. Gastrointest.* 16: 907-911.
- HAGEDORN, H. & JENSEN, N. 1935. Hørlige tælbæder (ill. besejningsbæder) blodsukker ved kaffinærferrugensid. *Hospitalvidende* 78: 1193-1201.
- HONG, S. S., CHIN, D. S. & HURL, K. B. 1961. Influences of hexamethonium and some dietary factors on human pancreatic and bile secretion. *J. Appl. Physiol.* 19: 810-814.
- HUNT, J. & CASH, R. 1975. Energy density of food, gastric emptying and obesity. *Lancet* ii, 905-906.
- HUNT, J. & STUBBS, B. F. 1975. The volume and energy content of meals as determinants of gastric emptying. *J. Physiol.* 225: 209-225.
- HYDÉN, B. 1955. The recovery of polyethylene glycol after passage through the digestive tract. *Institute of Animal Physiology* 22: 411-424.
- JENDRASSIK, K. L. & GROF, D. 1938. Vereinfachte photometrische Methoden zur Bestimmung des Bilebilirubins. *Biochem. Z.* 797: 81-89.
- JOHANSSON, C. 1974. Studies of gastrointestinal interactions. *Scand. J. Gastrointest.* 9: Suppl. 28.
- JOHANSSON, C. & EKELUND, K. 1976. The relation between body weight and the gastric and intestinal handling of peroral caloric load. *Gut* 17: 456-462.
- LAGERLÖF, H. O., JOHANSSON, C. & EKELUND, K. 1976. Human gastric and intestinal response to meals studied by multiple indicator dilution method. *Mi. Sui. J. Med.* 43: 1-98.
- NORBY, G. 1935. Om æmningens blod og urter. Levin and Munksgaard, Denmark.
- REIDER, H. P. 1939. Verbesserungen zur Methode der Enzymbestimmung mittels Ca (II) und Phenolreagenzien nach Folin-Ciocalteu. *Clin. Chim. Acta* 4: 733-740.

REFERENCES

- NEIL, H., NORTHFIELD, T., HOFFMAN, A. W. & SUMMERSKILL, W. 1974. Gastric emptying and secretion of bile acids, cholesterol and pancreatic enzymes during digestion. *Mayo Clin. Proc.* 49: 851-860.

ges in cerebral blood flow during hyperventilation $^{18}O_2$ -breathing measured transcutaneously in humans bidirectional pulsed ultrasound doppler velocitymeter

J. HAUGE, M. MARIANNE THORESEN and LARS WALLOE

of Physiology University of Oslo, Norway

HAUGE, A. THORESEN M. & WALLOE, L. Changes in cerebral blood flow during hyperventilation and CO_2 -breathing measured transcutaneously in humans by bidirectional pulsed, ultrasound doppler blood velocitymeter. *Acta Physiol Scand* 1980 110 167-173. Received 30 Jan. 1980 ISSN 0001-4772. Institut of Physiology University of Oslo Norway

We have used bidirectional pulsed ultrasound doppler system which measures the instantaneous mean velocity across the lumen of a blood vessel in order to determine the relationship between alveolar $P_{A_{CO_2}}$ and blood flow in the four arteries supplying the brain in humans. Both high and low $P_{A_{CO_2}}$ -values were explored. Six subjects, 3 males and 3 females (22-40 years) were studied by use of this non-invasive technique. To increase the $P_{A_{CO_2}}$ the subjects were breathing 4.6 and 8.0% CO_2 in air. $P_{A_{CO_2}}$ was reduced by voluntary hyperventilation down to chosen end-expiratory P_{CO_2} values of about 2.2 kPa. We found a linear relationship between arterial blood flow expressed as percentage of control level and $P_{A_{CO_2}}$ in the range from 3.3 to 7.3 kPa. At the very lowest $P_{A_{CO_2}}$ -values levelling off of the response with flow values of 40 to 45% was observed. The CO_2 -reactivities in the 6 persons varied between 28.1 and 30.0%/kPa. The time courses and the magnitude of the flow response were similar in all four arteries.

different method have been used to measure blood flow in the experimental animal. Few of these methods can be applied in man, the most important today being the electric, the Xe-injection and the Xe-inhalation. The electromagnetic flowmeter (review by Greenfield & Mills 1972) can measure the in-vivo blood flow through a cross section of the internal carotid or vertebral arteries. The advantage of this method is of course that it is surgically exposed artery with the patient under general anesthesia. It is therefore only applied during certain surgical operations, and only on quite specific indications. The ^{133}Xe arterial injection method is in much more frequent use on patients (e.g. Tomura et al. 1976; Hachinski et al. 1977; Hachinski et al. 1977). This method does not measure the instantaneous blood flow but the average flow over some minutes. The uncertainty in each single measurement is

considerable and the risk connected with the carotid or femoral puncture and the discomfort of the patient seriously limit the number of measurements one can obtain from each person. In practice there is a time span of at least 20 min between each injection. The method does give information about the regional distribution of flow (Olesen et al. 1971; Ingvar & Lassen 1977). However it cannot be applied to the vertebral artery. During the last few years the ^{133}Xe inhalation method has been improved (Obrist et al. 1975; Meyer 1978). This method is noninvasive but the clearance curves are contaminated by radioactivity from the scalp and other extracranial sources.

A fourth method which potentially could be useful on patients is the ultrasound doppler method. In their earliest papers Baker, Franklin, Rushmer and collaborators (1959-1961) indicated the possibility of developing equipment which could measure volumetric flow. Due to practical difficulties and to the

operating principles implemented in the instruments available commercially these instruments have not yet been able to do this.

In our laboratories we have developed a bidirectional pulsed ultrasound doppler system ("UNIDOP") which measures the instantaneous mean velocity across the lumen of a blood vessel (Haugland & Eriksen 1980, Wille 1977). As long as the cross sectional area of the vessel is constant this mean velocity is proportional to flow. The performance of the instrument used transcutaneously has been compared to simultaneous electromagnetic flow measurements on the same common carotid and femoral arteries in dogs (Guldvog et al. 1980). The similarity in flow pulse shape was good.

As a result of these encouraging observations we wanted to explore the possibility of using the instrument to measure cerebral blood flow in humans. For our first experimental series we wanted to choose a physiological situation where the flow response to a certain degree is known from the use of other methods. We have chosen to use the flow response to step changes in alveolar P .

The relationship between alveolar P (P_A) alternatively arterial P , (P_a , $P_{a,i}$) and cerebral blood flow has been investigated by many authors both in animals (e.g. Reivich 1964, Harper & Glass 1965, Grubb et al. 1977) and in humans (Kety & Schmidt 1946, 1948, Severinghaus & Lassen 1967, Tominaga et al. 1976). In most experimental animals the blood flow to the brain is a sigmoid function of P_A with normal resting P values on the steepest part of the curve. The relationship in humans is of course not explored to the extreme P_A values and there is large scatter in the individual points obtained by the Xe injection method. In spite of these difficulties most authors describe a sigmoid relationship also in humans (Kety & Schmidt 1948, Olesen et al. 1971, Tominaga et al. 1976) with a linear relationship in the neighbourhood of normal resting values. Our results obtained noninvasively are close to the results obtained by previous authors: the main difference is a more extended linear part in the middle of the curve.

A preliminary report on some of the results has been given at a meeting of The Physiological Society (Thoresen & Walloe 1979).

METHODS

The doppler velocitymeter UNIDOP used in the present investigation and the computer systems used for data

analysis have been described in detail elsewhere (Lund & Eriksen 1980, Wille 1977).

UNIDOP operates on two different frequencies 1.5 MHz and 6 MHz and can operate either in a wave mode or in a pulsed wave mode on either frequencies. The low frequency 1.5 MHz is used for measuring velocities in deep vessels and the high frequency is from 1 to 10 cm. The 6 MHz is used on more superficial vessels with diameter 1 mm and the depth range is from 0.2 to 3 cm.

UNIDOP contains sharp low-pass filters (1-pole filterworth) adjustable to the four different frequencies. Depending on the measurement of velocities expected one can choose among three (200, 400 and 700 Hz). These filters eliminate artefacts caused by vessel wall movements and other artefacts.

The velocitymeter is connected to an analog time-compression spectrum analyser in our set up the doppler signal is fed through a band pass filter 70 ms giving the relative intensity of all the present in the vessel at that point in time. It is to see the spectrum on a scope simultaneously with the audio signal from the loudspeaker. One can eliminate most of the noise without losing too much information-containing signal. Another use of the spectrum is to discriminate between different vessels according to the characteristic shape of their spectra.

To store the audio signal for later calculation UNIDOP is connected to a stereo cassette tape recorder (QVC 760 D). The doppler signal is recorded on one channel and the ECG and comments are recorded on the other channel. Both the tape-recorder and the velocitymeter are connected on line to a NORD computer system. For technical reasons we usually store the recordings on tape and play the tape back for later calculation on the computer. The computer calculates the spectrum, ms, using a fast Fourier transform program, and mean velocity from each spectrum (Wille 1977).

The whole cross section of the vessel is illuminated by a soundfield of approximately constant intensity (Guldvog et al. 1980). If one varies the cross sectional area of the vessel does not during the recording changes in the mean velocity proportional to changes in instantaneous flow. The computer also calculates the time average instantaneous mean velocity in one cardiac cycle triggered by the ECG. This average mean velocity is proportional to the average volume flow during the diastolic cycle.

The experiments reported in this paper were performed on six subjects, 3 males and 3 females (22–40 years) who were normotensive and without any known disease. During the experiment the subject was in a quiet room with temperature $\pm 2-5^\circ\text{C}$ with a nose and breathing through a nonrebreathing apparatus connected to a suitable mouthpiece.

To increase the $P_{a,i}$ the subjects were breathing 8% CO_2 in air for about 4 minutes. To lower $P_{a,i}$ they were hyperventilating voluntarily down to a venous end-expiratory $P_{a,i}$ value. During the hyper-

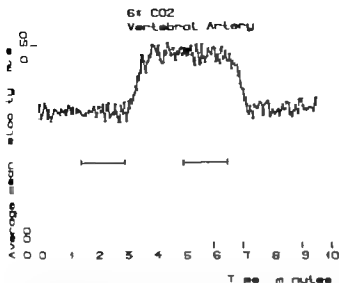


Fig. 1. Average blood velocity in the right vertebral artery during CO_2 breathing. The figure shows the recording of blood velocities in the right vertebral artery. Each point represents the time average over one cardiac cycle of the cross-sectional mean velocity in the vessel. The figure displays the values for each third cardiac cycle. As long as the cross-sectional area of the vessel is constant, the average velocity is proportional to flow (see Methods). The right vertebral artery was measured on a 26-year-old male subject with a 1.5 MHz transducer at 4 cm depth behind the mastoid process of the skull. Mean velocity in the marked-out 1.5 min period prior to the breathing of gas was 0.3291 m/s , SD $\pm 0.0121 \text{ m/s}$, giving $\text{CV} = 0.0369$. The corresponding values during gas breathing were 0.4740 m/s , $\pm 0.0144 \text{ m/s}$, and 0.0340 . Thus, in this experiment the flow increased to 144% of the resting value during gas breathing. $P_{\text{A-tO}_2}$ increased from 5.48 to 6.97 kPa.

periment, the end-expiratory $P_{\text{A-tO}_2}$ values were continuously read aloud to the subjects, and they tried to alter their breathing to keep a certain $P_{\text{A-tO}_2}$. Each they tried to keep 2% of this value. End-tidal $P_{\text{A-tO}_2}$ was continuously monitored with an infrared CO_2 analyzer (LKB-2, connected to Hewlett-Packard poly-

graph). Flow measurement on the internal carotid artery. A 1.5 MHz transducer was held with approximately 45° to the direction of the artery beneath the angle of the jaw, the depth varying between 1.0 and 1.7 cm. Measuring the vertebral artery, the 1.5 MHz transducer was held at the mastoid process pointing to the bottom of the vertebral foramen to catch the artery between atlas and occipital. The depth varied between 3.5 and 5 cm. The angle between the transducer and the artery is however variable, because of the large individual variations in the shape of the artery (Luzzati 1974).

Since CO_2 breathing only causes slight hypertension, the changes in blood pressure per se does not affect cerebral blood flow (Tatebayashi et al. 1976), we have not varied the blood pressure systematically during the experiment.

RESULTS

In one series of experiments the content of CO_2 in the inspired air was increased in one step from 0 to 4.6 or 8.9% and the resulting blood velocities in one of the internal carotid or vertebral arteries were measured. Fig. 1 shows the results from one such experiment. As illustrated in the figure, the blood velocities in a steady state situation only vary within narrow limits. Typically the coefficient of variation is less than 0.05. As the gas mixture is switched on, the blood velocities increase through a short transient period to a new steady state. The magnitude of the increase is larger with higher CO_2 content in the air. When the gas mixture is switched off, the blood velocities return to resting values within one minute.

The time course and the magnitude of the flow response were similar in all four arteries supplying

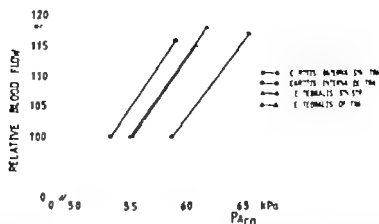


Fig. 2. Relative blood velocity in each of the four vessels supplying the brain as function of P_{aCO_2} . Average P_{aCO_2} in two steady state periods of 1.5 min duration approximately 1 min apart one immediately before breathing 4% CO_2 in air and one during such breathing in four different experiments. Ordinate: Percentage change in blood flow in each of the four vessels supplying the brain in one subject due to the breathing of 4% CO_2 . The four vessels were measured on four different days in the same subject as in Fig. 1. The CO_2 reactivity in these four vessels varied between 22.5 and 38.5 %/kPa (3.4 and 3.8 %/mmHg).

the brain. Fig. 2 displays the results from four experiments on one person, one from each artery. Although the figure reveals large differences in resting P_{aCO_2} explained by the fact that the experiments were performed on four different days, the

CO_2 -reactivities (that is, the slopes of the lines, e.g. % flow change per CO_2 pressure unit) are similar. It was always found in our experiments on normal subjects that the four arteries supplying the brain had equal CO_2 -reactivity.

In another series of experiments the P_{aCO_2} was lowered by voluntary hyperventilation down to a certain preset P_{aCO_2} value. Fig. 3 shows a recording from a typical experiment on the right internal carotid artery. In this experiment the blood flow displayed an initial sudden decrease followed by a slower decrease. The detailed time course of the experiments depended on how the subject was on the pre-determined P_{aCO_2} value.

Fig. 4 displays the steady state values obtained from all experiments performed on one subject both during CO_2 -breathing and during hyperventilation. To eliminate the variation from one experiment to another in resting P_{aCO_2} , we have added the difference between 5.33 kPa and the measured P_{aCO_2} both to the high and the low P_{aCO_2} values in each experiment. The resulting normalized relationship between P_{aCO_2} and cerebral blood flow is shown in Fig. 5A. There is a linear relationship between the P_{aCO_2} range from 3.3 to 7.3 kPa (25 to 55 mmHg). At very low P_{aCO_2} values (less than approximately 3.0 kPa) the figure suggests a level off of the flow response with relative flow values in the order 40 to 45 %.

The results from the other persons investigated

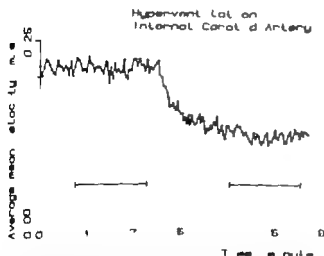


Fig. 3. Blood flow to the brain during hyperventilation. The tracing shows the blood velocity in the right internal carotid artery during a hyperventilation experiment. The measurements were obtained with a 6 MHz transducer 1.5 cm depth beneath the angle of the jaw on the same subject as in Figs. 1 and 2. Mean velocity in the 1.5 min period prior to hyperventilation was 0.127 m/s and CV was 0.0358. The corresponding values during steady state hyperventilation were 0.1198 m/s and 0.0576. P_{aCO_2} decreased from 5.6 kPa to 3.9 kPa.

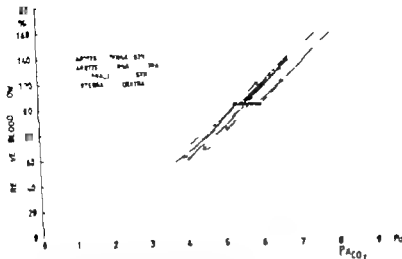


Fig. 4. Relative blood flow as function of P_{ACO_2} . Abscissa and ordinate as in Fig. 3. The figure displays the results from all experiments performed on one person. The results shown in Figs. 1, 2 and 3 can also be found in this figure.

similar. Altogether six persons have been studied in comparable detail. They all displayed a linear relationship between P_{ACO_2} and flow. λ_1 reactivity was equal for all six, well within experimental accuracy. Fig. 5B, C and D show relative blood flow as function of P_{ACO_2} for three of the persons which has not been used in the other figures. The four panels in Fig. 5 illustrate the typical individual differences. At low values there were larger variations in relative flow than in other parts of the curves, due to coarse measurements when the subjects were respirating violently and probably also to small differences.

DISCUSSION

Results presented in this paper show a simple relationship between P_{ACO_2} and average blood velocity in all four arteries supplying the brain. The relationship is linear over most of the investigated range, only levelling off for very low P_{ACO_2} values. Not only are the results highly reproducible for each person, but the results are also similar for all six persons investigated, with the same shape and equal slope of the straight lines. The CO_2 -reactivities found in our 6 persons varied from 28.1 and 30.0 %/kPa (3.75–4.1 %/mmHg) with a median of 29.4 %/kPa (3.97 %/mmHg). The conversion from average blood velocity to

relative blood flow rests on the assumption of a constant cross-sectional area of the artery throughout all measurements made on that artery. This assumption is of course questionable. Changes in the cross-sectional area of a vessel could be caused either by changes in blood pressure or by changes in tension of the smooth muscle in the vessel wall. Changes in area caused by changes in blood pressure are probably not important in our experiments. This conclusion is valid both for the area changes in each cardiac cycle, which is less than 11 % in humans (Olsson 1974; Schabert 1978) and for long term changes, since blood pressure shows only minor variations with changing P_{ACO_2} (Tomimaga et al. 1976; Olesen et al. 1971; Kety & Schmidt 1948). Less is known about the effect of changes in P_{ACO_2} on the smooth muscle in the wall of the arteries. While we tend to believe that also this effect must be small, we have only indirect evidence: (1) There are no reports in the literature of effects of changes in P_{CO_2} on the tension of isolated strips of vascular smooth muscle, while such effects of changes in P_{O_2} are well known. (2) The CO_2 -reactivity values found in our experiments are in good agreement with reactivity values found by others. Fig. 3 in Kety & Schmidt (1948) shows a CO_2 -reactivity in the neighbourhood of 3.75 %/mmHg around resting values and an increasing reactivity with higher P_{ACO_2} -values. They used the N_2O inhalation method. Tomimaga et al. (1976) using the Xe injec-

like to thank Morten Kjernes for help with the text, and Kari Læstøl, Arild Nyl and Jan Marksten for comments on the manuscript. M. T. was a postgraduate fellow ship from The Norwegian Council for Science and the Humanities. Financial support was also provided by The Royal Norwegian Scientific and Industrial Research and by Varg-

REFERENCES

- JOHNSON, R. H. & GALBO, J. E. 1963 Effects of carbon dioxide on cerebral hemodynamics in normal man and in cerebro-vascular disease studied by direct injection of radio-contrast. *Circ Res* 13 446-456.
- JONAS, D. BAKER, D. ELLIS, R. & RUSH, R. 1979 A pulsed ultrasonic flowmeter. *IEEE Med Electronics* ME-6, 204-206.
- JONAS, D. SCHLEGEL, W. & RUSHMER, R. F. 1978 Blood flow measured by Doppler frequency shift back-scattered ultrasound. *Science* 134 364-365.
- WILLIS, C. D. COLACINO, J. M. & ERIKSEN, K. 1977 Effect of arterial carbon dioxide on cerebral blood flow in ducks. *Am J Physiol* 232 H596-H601.
- OGSTAD, K. J. & THORESEN, M. 1980 Blood flow in arteries determined noninvasively by an ultrasonic Doppler velocity measurement compared to electromagnetic measurements in exposed vessels. *Acta Physiol Scand* 109 211.
- NEEL, V. C., OLESEN, J., MORRIS, J. W., EISEN, B., ENEVOLDSEN, E. & LASSEN, N. 1977 Cerebral hemodynamics in migraine. *Can J Neurol Sci* 4 245-249.
- JOHNSON, R. H. & GLASS, H. L. 1965 Effect of alteration of the arterial carbon dioxide tension on the blood flow through the cerebral cortex at normal and low arterial blood pressures. *J Neurol Neurosurg Psychiatr* 32 449-452.
- LAND, K. & ERIKSEN, M. 1980 A heterodyne beat method blood velocity-meter. *Med Biol Eng* 18 1-6.
- JOHNSON, D. H. & LASSEN, N. A. 1977 Measurement of regional cerebral blood flow. In: *J Neurol* 11 107-119.
- KETY, S. S. & SCHMIDT, C. F. 1948 The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. *J Clin Invest* 27 484-492.
- LUZSA, J. 1974 X-ray anatomy of the vascular system. p. 150. Butterworths, London.
- MEYER, J. S. 1978 Improved method for noninvasive measurement of regional cerebral blood flow by ^{133}Xe inhalation. *Stroke* 9 205-210.
- MILLS, C. J. 1972 Measurement of pulsatile flow and flow velocity. In: *Cardiovascular fluid dynamics* (ed. D. H. Bergel), vol. 1, pp. 51-90. Academic Press, London.
- OBRIEN, W. D., THOMPSON, H. K., RANO, H. S. & WILKINSON, W. E. 1974 Regional cerebral blood flow estimated by ^{133}Xe inhalation. *Stroke* 5 245-254.
- OLESEN, J., PAULSON, O. B. & LASSEN, N. A. 1971 Regional cerebral blood flow in man determined by the initial slope of the clearance of intrarterially injected ^{133}Xe . *Stroke* 2 519-540.
- OLSON, R. M. 1974 Human carotid artery wall thickness, diameter and blood flow by a noninvasive technique. *J Appl Physiol* 37 955-960.
- PALMER, M. J., THOMAS, D. J., MACGILLIVRAY, B. B., DU BOULAY, G. H., MARSHALL, J., RUSSELL, R. R. & SYMON, L. 1977 Variations in mean cerebral blood flow under acrobatic rest and during cortical activation. *Stroke* 8 269-271.
- REIVICH, M. 1964 Arterial P_{CO_2} and cerebral hemodynamics. *Am J Physiol* 206 25-35.
- SCHABERT, A. 1978 Separation of time-dependent and time-independent terms in relation to pressure and diameter and of pressure and flow velocity in arteries. In: *The arterial system* (ed. R. H. Baker and R. B. St. Louis), pp. 89-94. Springer Verlag, Berlin.
- SEVERINGHAUS, J. W. & LASSEN, N. A. 1967 Step hypoxemia to separate arterial from tissue P_{O_2} in the regulation of cerebral blood flow. *Circ Res* 20 272-278.
- THORESEN, M. & WALLOE, L. 1979 Changes in cerebral blood flow during hyperventilation and CO_2 -breathing measured by ultrasound. *J Physiol* 298 53-54 P.
- TOMINAGA, S., STRANDGAARD, S., UEMURA, K., ITO, K., KUTSUZAWA, T., LASSEN, N. A. & NAKAMURA, T. 1976 Cerebrovascular CO reactivity in normotensive and hypertensive man. *Stroke* 7 507-510.
- WILLIS, S. 1977 A computer system for on-line decoding of ultrasonic Doppler signals from blood flow measurement. *Ultrasonics* 15 226-230.

thermoregulatory responses to tyramine in the pigeon

J. C. GEORGE, E. D. STEVENS and S. SAARELA

Department of Zoology Zoophysiological Laboratory University of Oulu, Finland and Department of Zoology University of Guelph, Guelph, Ontario Canada

HISKA J. C. GEORGE, J. C. STEVENS E. D. & SAARELA S. Thermoregulatory responses to tyramine in the pigeon. *Acta Physiol Scand* 1980, 110 175-180. Received 21 Jan. 1980. ISSN 0001-6772. Department of Zoology Zoophysiological Laboratory University of Oulu Finland and Department of Zoology University of Guelph, Guelph, Ontario, Canada.

Intravenous injections of different doses of tyramine induced hypothermia in the pigeon in the cold and moderate hyperthermia in the warm environment. The hypothermia was correlated with dose-dependent decline in shivering. Hyperthermia was attributed to the chemo- and isotropic effects of tyramine. The indirect stimulatory effect of noradrenaline (NA) at the tissue level were studied. Pretreatment with α -methyl-para-tyrosine (α -MPT) and blocking α -adrenoceptors with phentolamine diminished the hypothermia induced by tyramine in the cold. The results obtained indicate that the release of endogenous NA stimulated by tyramine might result either in hypothermia or hyperthermia, thus resembling similar effects obtained with exogenous NA in birds.

Key words: Tyramine, noradrenaline, α -methyl-para-tyrosine, phentolamine, hypothermia, hyperthermia, electromyographic activity.

The information is available on the effects of tyramine on thermoregulation in birds. Key & Mar (1967) observed that although neither intraperitoneal nor intramuscular injection of tyramine (100-250 μ g per kg) had any effect on behavioural and electrical activity in 128 day old chicks yet it produced behavioural and electrocortical effects. El-Habib et al. (1970) noted in 5 and 9 week old cold-adapted chickens that intramuscular injection (200 mg/kg) decreased both oxygen consumption (VO_2) and body temperature but induced electromyographic activity. It has been suggested that tyramine releases the endogenous noradrenaline (NA) from its storage sites in nerve endings. Since the liberated NA is subjected to intraneuronal degradation before reaching the extraneuronal space (Trendelenburg 1972; Brandão et al. 1978) a very small amount of NA escapes degradation. Nevertheless, tyramine has repeatedly been shown to produce sympathomimetic effects by its action in preventing the reuptake of released neurotransmitters (see Trendelenburg 1972). α -Methyl-para-tyrosine (α -MPT) is a potent

competitive inhibitor of tyrosine hydroxylase enzyme (see Nagatsu et al. 1964; Udenfriend et al. 1965) and it is widely used to suppress the endogenous catecholamine synthesis (e.g. Spector et al. 1965; Saarela et al. 1977; Widerlov 1979). In our previous study (Saarela et al. 1977) α -MPT was shown to abate significantly catecholamine synthesis in the temperature stressed pigeons 4 h after the administration.

The present investigation was undertaken in order to study the effect of endogenous NA on body (T_b) and foot (T_f) temperatures, electromyographic (EMG) activity, heart rate and blood pressure at three different ambient (T_a) temperatures after the stimulation of its release with tyramine. In addition, the effects of tyramine after blocking catecholamine synthesis with α -MPT and blocking α -adrenoceptors with phentolamine were examined.

MATERIALS AND METHODS

Adult pigeons of both sexes weighing 330-395 g. were obtained from commercial supplier and were acclimated in individual cages in a thermostatically controlled room at 22°C at least for a period of one month prior to the com-

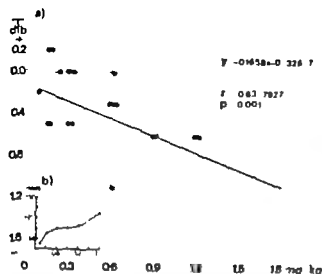


Fig. 1a Dose response of the mean maximal fall in body temperature (T_b) to iv injection of different doses of tyramine in the cold. The linear regression is fitted to all points by the method of least squares. Altogether 44 expts were performed with 22 pigeons.

Fig. 1b Mean time to maximal fall in T after different dose of tyramine (mg/kg).

commencement of experiments. The lighting regimen was 12 h of light and 1 h of darkness. Relative humidity was maintained at approximately 30%.

All measurements were performed in a darkened chamber maintained in a regulated water bath either at 4°C or 30°C as described by Hissa et al. (1980a).

The pigeons were kept unrestrained in the chamber and were given 1–2 h for adjustment before injections. All injections were made between 11.00 and 13.00 hours.

For measuring cloacal and foot temperatures, shivering and heart rate, the methods have earlier been described by Hissa et al. (1980a). Briefly, the temperatures were recorded continuously throughout the expt on a potentiometer and displayed with a Grass penrecorder. Muscle potentials were recorded from pin electrodes inserted into the breast muscle. Shivering was measured as described earlier by Hissa et al. (1980b) by integrating electromyograms (EMG) and feeding the amplified and rectified po-

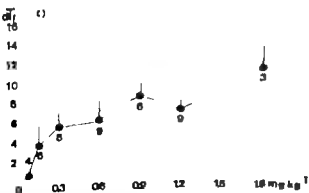


Fig. 1c Dose-response curve of T to iv tyramine in pigeons at 4°C. Vertical bars indicate \pm S.E. of mean. Number of animals used are given at each point.

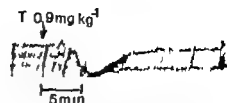


Fig. 2 A typical tracing of EMG integration of tyramine (0.9 mg/kg) in the cold.

tentials (time constant 0.04 s) with a Grass 7P10 B Integrator triggered to reset every 1/10 height of the record reflected the total shivering put was displayed with a Grass penrecorder.

For iv injection of tyramine or saline technique as described by Hissa et al. (1980a). For intrahypothalamic injections of tyramine a guide cannula was implanted stereotactically in the anterior hypothalamus as described earlier (Rauvengier 1974). The modified stereotaxic (Karten & Hodon 1967; Pyörälä et al. 1977) set mm anterior from the intra-aural line, 1.0 mm below the mid-line and 9.0–10.0 mm below the nuchal varium.

The direct blood pressure was recorded can be cannulated of the brachial artery and the Physiotransducer (Altech MSD 10-5). The temperature transducer was displayed with a Radiodet DP-4 shlin) recorder. Tyramine was administered in the iliac vein. ECG was recorded with a Phy (Schwarzer Varioscop V8-2) and EMG was by the electrodes fed into the differential amplifier, a band-pass filter. The high and low-cut frequencies of the filter were 500 Hz and 10 Hz respectively. amplified and filtered signals were rectified and displayed with a Radiodet DP-6 recorder.

In studying the effect of iv tyramine (10 µg) of injection at 20°C on the endogenous catecholamines, plasma adrenaline (A) and noradrenaline (NA) levels were assayed spectrophotofluorimetrically according to the method of Diamant & Byem (1978) modified by Hottola et al. (1978).

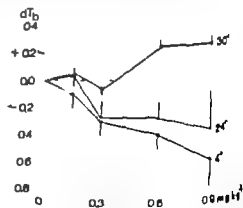
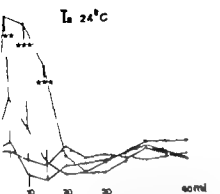


Fig. 3 Comparisons of changes in T after different doses of tyramine (14°C (●—●), 24°C (▲—▲) and 30°C (□—□)) in the pigeon. Each symbol represents 4–6 separate measurements. Vertical bars indicate \pm S.E. of mean.



The time-course of changes in heart-rate (dHr) of pigeons at 24°C. Δ - Δ 0.1, \bigcirc - \bigcirc 0.3 ($N=4$) 0.6 ($N=5$) and \bullet - \bullet 0.9 ($N=5$) mg/kg of T at 24°C. Values indicate positive and negative changes in comparison to its preinjection values. Bars \pm S.E. of mean. $***P<0.001$.

Tyramine HCl (Sigma) and noradrenaline (NA) (not bazaratrate, Sigma), Phenolamine (Methylone, CIBA) and α -methyl-para-tyrosine (Sigma) were dissolved in 0.85% w/v NaCl (saline) freshly every day. Control pigeons received the same volume (0.1-0.3 ml or 1 μ l) of saline or intracerebral injections. All doses of T are expressed in terms of base.

Values are expressed as the mean \pm S.E. of statistical analysis as performed with Student's t -test for paired comparisons. Regression lines and correlation coefficients are calculated according to the method of least squares. Results occurring with change of less than 0.05 were considered statistically not significant.

LTS

Experimental procedures

To show the effect of T injections of various doses of tyramine on T and T in the cold, a response curve was obtained by recording the changes in cloacal temperature for each dose and described by the method of least squares. At this T_a , the mean T was $39.6 \pm 0.1^\circ\text{C}$ and T $13.2 \pm 0.9^\circ\text{C}$ ($N=77$) at the beginning of the experiment. Saline injection had no significant effect on the level of the variables measured. The T was correlated with an increased heat loss indicated by the elevated T following the injection and is correlated with decrease in heat production indicated by the decreased EMG of shivering. It is noted that a significant depression of T (mean

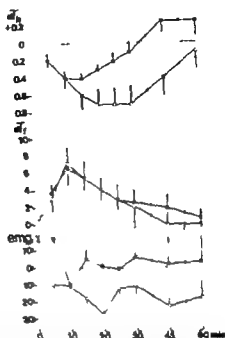


Fig. 3 Time course responses of the body temperature expressed as mean cloacal changes (dT_c , °C), foot temperature (dT_f , °C) and electromyographic activity (EMG in percentage) to tyramine 1-2 mg/kg in the pigeon pretreated with α -methyl-para-tyrosine (250 mg/kg) either 1 h (\square - \square) or 4 h (\blacksquare - \blacksquare) before tyramine injection at 4°C. Each point is the mean maximal change over the preinjection value. Vertical bars indicate \pm S.E. of mean. $P<0.05$.

-0.26°C) was observed at a dose of only 0.3 mg/kg ($P<0.001$) and that the most dramatic effects of T also occurred at low dose levels (T increased 5°C at a dose of 0.3 mg/kg). The pattern of integrated electromyographic activity after a single i.v. injection of 0.9 mg/kg tyramine is shown in Fig. 2. In general, the first sign of the decline in shivering did not occur earlier than 3-5 min of the injection. As shown in Fig. 3 the thermoregulatory response to tyramine depends on the ambient temperature. At 30°C the highest doses (0.6-0.9 mg/kg) caused a moderate rise in T_c . At 24°C a slight fall in T was observed with 0.3-0.9 mg/kg of tyramine (not dose-dependent). However at 4°C a clearly dose dependent drop in T was substantiated.

In analysing the possible causes for an elevated T in the warm environment in response to tyramine the chronotropic and inotropic effects at 30°C and 24°C , must be considered. The time course of the changes in heart rate (beats/min) at 24°C are

Table 1 Effect of i.v. injection of tyramine (1.2 mg/kg) after pretreatment with α -methyl-p-tyrosine (α -MpT) and phentolamine (Ph) on the mean maximal body (T_b) and foot (T_f) temperature change

Pretreatment Time Dose	Saline		α MpT 2 h 50 mg/kg		α MpT 4 h 50 mg/kg		Ph 30 min 3 mg/kg	
dT_b	-0.7 ± 0.13	6	-0.7 ± 0.19	6	-0.4 ± 0.07	6	-0.1 ± 0.05	6
dT_f	$+7 \pm 0.97$	9	$+6.2 \pm 1.83$	6	$+7 \pm 0.8$	6	$+3.5 \pm 2.5$	6

4 h α MpT vs saline not significant Ph vs saline $P < 0.01$

illustrated in Fig. 4. As depicted tyramine produced dose related effects on heart rate. Significance values are given in the figure. With the greatest dose (0.9 mg/kg) the elevation of heart rate was during the first 5 and 10 min (135 ± 14 and 178 ± 17 beats/min respectively above the preinjection level of 162 ± 17 beats/min). Quantitatively and qualitatively corresponding elevation in the heart rate from 133 ± 8 beats/min to 284 ± 64 beats/min was attained at 30°C too.

Pretreatment of the pigeon with α MpT (250 mg/kg) 2 or 4 h before tyramine injection reduced the fall in T_b only after 4 h of the tyramine injection (Fig. 5 Table 1). No differences in foot temperature were obtained. The normally produced decline in shivering with tyramine (0.9 mg/kg) was markedly reduced after pretreating the animals with α MpT 4 h before tyramine administration (Fig. 5).

As shown in Table 1 phentolamine (3 mg/kg) caused a significant antagonism to tyramine ($P < 0.1$).

To study the effect of tyramine on the blood pressure 3 dose runs of 0.8, 1.6 and 3.2 mg/kg at 10°C were performed. With the two smaller doses the systemic blood pressure was elevated by only 8–14 mmHg whereas the 3.2 mg/kg dose of tyramine was sufficient to cause an increase of 55 mmHg in 7 min of injection. This was correlated with a concomitant decline in shivering to 55% of the preinjection level.

Tyramine when administered i.v. at 1.2 mg/kg produced a marked increase in plasma level from 0.36 ± 0.05 to $0.51 \pm 0.14 \mu\text{g}/100 \text{ ml}$ (not significant). Plasma adrenaline was rising 0.47 ± 0.70 before and $0.45 \pm 0.18 \mu\text{g}/100 \text{ ml}$ after the treatment.

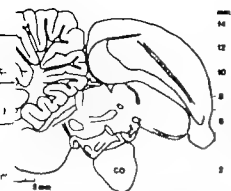
Table 2 shows the mean maximal rectal T_b and T_f in the pigeon following intrahypothalamic injections of noradrenaline or tyramine in the cold environment. The hypothermic response to NA ($5 \mu\text{g}/\mu\text{l}$) was similar to that documented in the pigeon in similar circumstances (Hissa & Rautenberg 1974). No thermoregulatory responses to tyramine ($10 \mu\text{g}/\mu\text{l}$) were obtained after injection in the same area. The schematic illustration of the injection area is shown in Fig. 1.

DISCUSSION

Since our present results with tyramine are compatible with those obtained by others with intracerebral injections of NA in birds (e.g. Allen & Freeman 1967; Freeman 1970; Hissa & Palokangas 1975; Hissa et al. 1975; Pyörälä et al. 1976) both in cold and warm environments they tend to confirm that these effects are attributable to the cold release of NA. Actually a slight elevation in plasma NA and A levels were demonstrated after tyramine injection. Tyramine like NA abolished shivering and elicited vasodilatation in the pigeon in the

Table 2 Differences in the effects of intrahypothalamic injections of noradrenaline (NA $5 \mu\text{g}/\mu\text{l}$) and tyramine (Ty $10 \mu\text{g}/\mu\text{l}$) in the same locus of the hypothalamus at 4°C . 11 measurements with 8 birds

	Mean max deviations in T ($^\circ\text{C}$)	N	Time (min)	Mean max deviations in T ($^\circ\text{C}$)	Time (min)	N
NA	-0.8 ± 0.27	11	24 ± 1.4	$+0.2 \pm 0.55$	16 ± 2.1	11
Ty	-0.1 ± 0.09	11	12 ± 2.1	-0.1 ± 0.19	1 ± 0.8	11



Sagittal drawing through the pigeon brain as in the Aras of Karten & Hodon (1967) indicating here noradrenaline (5 µg/µl) and tyramine (10 µg/µl) unilaterally injected. CO = cerebellum optimum.

more the response to tyramine was at-
tended after inhibition of the endogenous sym-
- NA with MPT in a warm environment
- raised as with NA injections. T was slight-
- ly elevated along with an increase in heart rate (see
- Allen et al 1976). Likewise the observation that
- the blockade abolished almost completely
- the thermoregulatory effect of tyramine is in accord with
- obtained in birds with NA and α -blockades
- & Marley 1967 Hissa et al 1973)
- total absence of thermoregulatory response
- after intrahypothalamic administration of
- NA could be due to the small dose injected
- indicated by the results obtained with intraven-
- ous injections of tyramine in the cat (Metcalfe &
- 1973). Probably tyramine after intra-
- hypothalamic injection did not reach a sufficient
- of neurotransmitter compartments in the
- vesicles to manifest the effects. Further-
- more it is also possible that due to the immediate
- massive deamination of released NA there were
- adequate number of neurotransmitters available
- to maintain the postsynaptic sites of the neuronal
- activity.

Our results are at least partly at variance with
- of El-Hadwani et al (1970) who found en-
- dogenous EMG activity after tyramine injection in the
- rat. Paradoxically however both $\dot{V}O_2$ and T
- were reported to be decreased. Apart from being
- species differences, we find no other explanation
- for this discrepancy.

Several schemes could be envisaged to ac-
- count for the inhibition of shivering after tyramine

administration one that appears most plausible is
- what is indicated by the recent findings of Marshall
- & Stoner (1979) with the rat and with the pigeon
- observed in our laboratory (Hohtola et al. 1980). A
- very close correlation between the decreased or
- increased blood pressure changes and abolishment
- of shivering was obtained. In the present work
- tyramine was found to produce an increase in blood
- pressure as well which might result in the changes
- in baroreceptor reflexes leading to the abolishment
- of shivering, as observed after i.v. NA injections in
- the pigeon (Hohtola et al 1980).

The neurogenic reflex vasodilatation elicited by
- substances that increase blood pressure is well
- documented (see Wilkins & Bogner 1978). Such a
- mechanism is quite feasible and could explain the
- observed increase in foot temperature especially in
- the cold after administration of tyramine. The vas-
- odilatory effect of tyramine has also been dem-
- onstrated in mammals (Burn & Tainter 1931
- Dadiar et al 1977).

To summarize the present experiments tend to
- indicate that the effects obtained with tyramine
- both in warm and cold environment can be attrib-
- uted to its effect caused indirectly via endogenous
- release of NA. Furthermore our results support the
- original hypothesis of the role of NA in thermoreg-
- ulation of birds. There was a fall in T in the cold
- environment, which is at least partly due to the
- decline in shivering and increased heat loss. In the
- warm environment a slight elevation in T might be
- a result of the excessively stimulated cardiac activ-
- ity. That NA was indeed involved is supported by
- the results obtained in experiments performed with
- the animals after reduction of their catecholamine
- pools. The direct central effect either on shivering
- motor centre or the blood pressure regulatory
- centre remains to be elucidated by attempting to
- block the effects of tyramine with injections of an
- α -blocking agent centrally.

Our sincere thanks are due to M. V. Maltby for his excel-
- lent technical assistance. The financial support for this
- work was received from the Natural Sciences and En-
- gineering Research Council of Canada and the National
- Science Council of Finland.

REFERENCES

- ALLEN J. J. & MARLEY E. 1967 Effect of sym-
- pathomimetic and allied amines on temperature and
- oxygen consumption in chickens. *Br. J. Pharmacol.*
- *Chirother.* 31: 290-312.

- BRANDAO F, MONTTIRO J G & OSSWALD W 1978 Differences in the metabolic fate of noradrenaline released by electrical stimulation or by tyramine. Naunyn-Schmiedeberg's Arch Pharmacol 305: 37-40.
- BURN J H & TAINTER M L 1931 An analysis of the effect of cocaine on the action of adrenaline and tyramine. J Physiol (Lond) 71: 169-193.
- DADKAR N K, DOHADWALLA A N & DHAT TACHARYA B L 1977 The effect of tyramine on peripheral vasculature of the spontaneously hypertensive rat. J Pharm Pharmacol 29: 48-49.
- DIAMANT J & BYERS O B 1975 A precise catecholamine assay for small plasma samples. J Lab Clin Med 85: 678-693.
- EL-HALAWANI M F, WILSON W O & BURGER H E 1970 Cold acclimation and the role of catecholamines in body temperature regulation in male Leghorns. Poultry Sci 49: 61-67.
- ERFMEIAN D M 1970 Thermoregulatory mechanisms of the neonate fowl. Comp Biochem Physiol 33: 19-230.
- HISSA R & PAIOKANGAS R 1970 Thermoregulation in the tilmouse (*Parus major* L.). Comp Biochem Physiol 33: 941-953.
- HISSA R & RAUTENBERG W 1974 The influence of centrally applied noradrenaline on shivering and body temperature in the pigeon. J Physiol (Lond) 234: 41-435.
- HISSA R, GEORGE J C & SAARELA S 1980a Dose related effects of noradrenaline and corticosterone on temperature regulation in the pigeon. Comp Biochem Physiol 65C: 25-32.
- HISSA R, SAARELA S & PYÖRNILÄ A 1975 Thermoregulatory effects of peripheral injection of monoamines on the pigeon. Comp Biochem Physiol 51C: 23-241.
- HISSA R, STEVENS E D & GEORGE J C 1980b Propranolol inhibits shivering in birds. Comp Biochem Physiol (In press).
- HOHTOLA E, RINTAMAKI H & HISSA R 1978 Differential impairment of thermogenesis in the pigeon after chemical sympathectomy. Can J Physiol Pharmacol 56: 578-584.
- HOHTOLA E, SAARELA S & HISSA R 1980 Effects of blood pressure manipulation on shivering thermogenesis in the pigeon. Acta Physiol Scand 110: 77-83.
- KARTEN H J & HODOS W 1967 A store of the brain of the pigeon (Columba livia). Johns Hopkins Press, Baltimore.
- KEY B J & MARLEY E 1966 The effect of pathetic amines on behaviour and excitability of the chicken. Electroencephalogr 14: 90-105.
- MARSHALL H W & STONER E I 1972 Catecholaminergic receptors and shivering. J Physiol (Lond) 262: 77-94.
- MITCHELL G & MYERS R D 1975 Evidence concerning the role of brain acetylcholine in mammalian thermoregulation. J Physiol 251: 616-617.
- NAGATSU T, LEVITT N & UDENFRIEND S 1964 Tyrosine hydroxylase: the endoneurial tyrosine hydroxylase. J Biol Chem 239: 1917.
- PYÖRNILÄ A, HISSA R & SAARELA S 1975 Effect of adrenergic blocking agents on peripheral renally induced thermoregulatory response in pigeon at heat. Pflügers Arch 365: 167-178.
- PYÖRNILÄ A, LAHTI H & HISSA R 1977 Thermoregulatory changes induced by intracerebral injections of cholinergic substances in the pigeon. Neuropharmacology 16: 737-741.
- SAARELA S, HISSA R, HOHTOLA E & NEN E 1977 Effect of α -methyl-tyrosine on temperature stress on monoamine and catecholamines in the pigeon. J Thermal Biol 2: 121-128.
- SPECTOR S, SJÖERDAMA A & UDENFRIEND S 1965 Blockade of endogenous norepinephrine by α -methyl-tyrosine as inhibitor of tyrosine hydroxylase. J Pharmacol Exp Ther 147: 84-94.
- TRENDELENBURG U 1972 Classification of pathomimetic amines. In: Handb. der systematischen Pharmakologie (ed. H. Blaschko and E. W. Berg). New York: Springer-Verlag, vol. 13, pp. 346-366.
- UDENFRIEND S, ZALTZMAN NIRENBERG NAGATSU T 1965 Inhibition of peripheral tyrosine hydroxylase. Biochem Biophys Res Commun 14: 837-845.
- WIDERLÖF E 1979 Dose-dependent plasma levels of α -methyl-tyrosine (α -MT) and catecholamine turnover rates after two doses. J Neural Transm. vol. 44: 143-158.

Membrane and biochemical alterations after denervation during reinnervation of mouse skeletal muscle

ELLIN R. LIBELIUS I LUNDQUIST ■ TÅGERUD and ■ THESLEFF

Dept of Pharmacology University of Lund, Sweden

SELLIN L. C. LIBELIUS R. LUNDQUIST I. TÅGERUD S. & THESLEFF S. Membrane and biochemical alterations after denervation and during reinnervation of mouse skeletal muscle. *Acta Physiol Scand* 1980 110: 181-186. Received 21 Jan. 1980. ISSN 0001-6777. Department of Pharmacology University of Lund, Sweden.

Denervation of the extensor digitorum longus (EDL) muscle of the mouse by either nerve crush or nerve section produced a reduction of the resting membrane potential (E_m) alterations in the properties of muscle fibre action potential and the development of tetrodotoxin (TTX)-resistant action potentials. These changes in membrane electrical properties were accompanied by an increase in the endocytic activity of the muscle and an increase in the activities of the lysosomal enzymes cathepsin D and N-acetylglucosaminidase (NAGA). Reinnervation of muscle was indicated at 9 days after nerve crush by the presence of miniature end-plate potentials. The recovery of membrane electrical properties, beginning with the onset of reinnervation, were not temporally related. The E_m increased in two stages, an early rapid repolarization and a later slower repolarization. The muscle fibers were sensitive to the blocking action of TTX by 12 days after nerve crush whereas the rate of rise (dV/dt) of the action potential did not approach values of innervated muscles until 21 days. Reinnervation resulted in a decrease in endocytosis and decrease in the activities of cathepsin D and NAGA toward innervated values by 21 days after nerve crush. The results suggest that membrane alterations after denervation and during reinnervation may occur by endo- and exocytosis of membrane constituents and that the lysosomal system may play a role in the breakdown and/or recycling of these structures.

Key words: Denervation, reinnervation, membrane electrical properties, endocytosis, lysosomal enzymes.

Denervation of mammalian skeletal muscle produces alterations in the electrical properties of the muscle. Among these are a fall of the resting membrane potential (E_m), alterations in the muscle action potential and the development of axonal (TTX-resistant) action potentials (Thesleff 1961, Rodforn & Thesleff 1971, b). The changes in the membrane and the development of TTX-resistant action potentials were to depend on de novo protein synthesis after denervation. The alteration in the muscle fibre action potential appeared to be independent of new protein synthesis (Gramp & Thesleff 1972). Removal and insertion of membrane proteins occur by the process of endo- and exocytosis. The rate of endocytosis, as measured by the uptake of extracellular marker molecules, was shown to be increased in denervated skeletal muscle (Libelius et al. 1979). In addition, denervation is reported to result

in increased activities of lysosomal enzymes (Wernstock & Odice 1969) which may be involved in the break-down and/or recycling of membrane constituents.

The purpose of the present study was to correlate the changes in membrane electrical properties after denervation and during reinnervation with concomitant changes in the rate of endocytosis and the activities of lysosomal enzymes. Some of the results have been presented in preliminary communication (Selin et al. 1979).

METHODS

All experiments were conducted *in vitro* on the extensor digitorum longus (EDL) muscle of adult male NMRI mice. Surgical procedures were done using diethyl ether for anaesthesia. Chronic unilateral denervation was performed by removing 1-4 mm sections of the deep peroneal nerve about 10 mm from its entrance into the muscle. For re-

nervation studies a 1 mm section of the nerve was crushed in the same position using a fine tipped forceps. Control muscles were obtained from the contralateral unoperated limbs. At 3, 6, 9, 14 and 21 days after nerve crush or section the muscles were examined for electrophysiological properties, rate of uptake of macro-molecular tracer or lysosomal enzyme activities.

Electrophysiological properties At the various times after nerve crush or section the EDL muscles were excised under a continuous flow of oxygenated (95% O_2 -5% CO_2) Krebs-Ringer solution having the following composition in mM: NaCl 135, KCl 5, CaCl₂ 4, MgCl₂ 1, NaH₂PO₄ 1, NaHCO₃ 15, dextrose 11. The pH of the solution was 7.2-7.3. The muscles were pinned through their tendons stretched to approximately their resting lengths, placed in a heated ($30 \pm 1^\circ C$) chamber with a volume of 25 ml and suffused at a rate of 4 ml/min. After a 30 min equilibration period muscle fibres were examined for resting membrane potentials and action potentials. In addition miniature end-plate potentials were recorded from some fibres to indicate the presence of reinnervation. For these measurements glass microelectrodes filled with 3 M KCl and having tip resistances of $10-15 \times 10^6 \Omega$ were used. Amplification and recording of potential were made using standard techniques (Katz & Thoenen 1957). The procedure for recording action potentials is described in detail elsewhere (Sellin & Thoenen 1979).

Uptake of ¹²⁵I-mulin Animals were killed by cervical dislocation at various times following nerve crush or section. The EDL muscles were excised and mounted on perspex holders kept in an oxygenated Krebs-Ringer solution ($22^\circ C$) as described above but with mM CaCl₂. Two muscles from each of the two experimental conditions and the four corresponding control muscles were mounted on the same holder. After mounting the muscles were placed in an oxygenated incubation solution at $+37^\circ C$ containing $3.0 \mu Ci/ml$ of ¹²⁵I-mulin (The Radiochemical Centre Ltd, Amersham, England) and incubated for 40 min. During the incubation the solution was continuously bubbled with 95% O_2 , 5% CO_2 supplied through a peristaltic pump (flow rate ca 12 ml/min) to give standardized oxygenation and stirring conditions. After incubation the muscles were washed in Ringer solution at $+4^\circ C$ for 4 h with the solution being changed each hour. The muscles were then blotted on filter paper, weighed and solubilized in 0.7 ml Soluene-100 (Packard Instrument Company Inc, Downers Grove, Ill, USA) over night at room temperature. Radioactivity was measured by liquid scintillation counting using 10 ml of a toluene-³H-POP mixture. Counting efficiency was calculated from an internal standard with ³H-toluene (Packard Instrument Company Inc, Downers Grove, Ill, USA).

Determination of lysosomal enzyme activity Cathepsin D activity was measured at pH 4.0 with haemoglobin as the substrate slightly modified from Barrett (1977). EDL muscles were homogenized in 3 ml ice-cold 0.3 mol/l sucrose in an Ultra Turrax homogenizer (Jank and Kunzel, Staufen, Germany) to yield a homogenate of approximately 0.5% muscle tissue (wet weight). The incubation mixture (0.4 ml) contained 0.2 ml muscle homogenate and 0.25 mol/l sodium acetate buffer (pH 4.0) with 0.1% Triton X 100. The incubation was

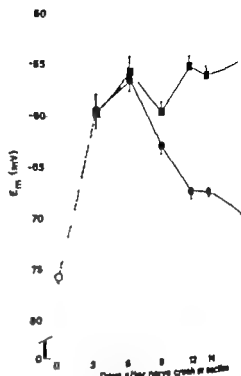


Fig. 1. Resting membrane potential (E_m) (mean $N = 4$ 706) in millivolts (mV) recorded from fibres in the extensor digitorum longus muscle of mouse as a function of days after crush (filled circles) or section (squares) of the deep peroneal nerve. The circle indicates the E_m of normally innervated muscle.

carried out for 120 min at $+37^\circ C$ and stopped by addition of 2 ml 3% TCA. After centrifugation, the clear supernatant was processed in the Folin-Lowry (Barrett 1972). Appropriate blanks and standards were included.

N-acetyl- β -D-glucosaminidase activity was measured after the muscles had been homogenized as above. Determination of N-acetyl- β -D-glucosaminidase activity was performed using 4-methylumbelliferyl- β -D-glucopyranoside as substrate as per Barrett (1972). In a mixture of 0.075 ml muscle homogenate and 0.05 mol/l sodium citrate (pH 4.5) in the presence of Triton X 100. The reaction was carried out for 30 min at $+37^\circ C$ and terminated by the addition of 2.5 ml glycine buffer (pH 10.6). The fluorescence was measured in an Aminco Bowman spectrofluorometer with excitation wavelength of 360 nm and an emission wavelength of 450 nm. Appropriate blanks and standards were included.

Protein determination was performed according to Lowry et al. (1951).

RESULTS

Electrophysiological properties Denervation of the extensor digitorum longus (EDL) muscle of the mouse produced significant alterations in the

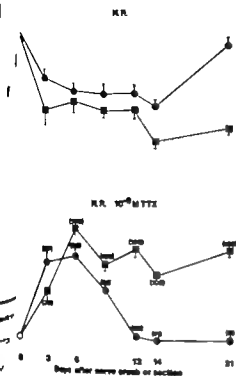


Fig. 1. Rate of rise (mean \pm S.E. $N = 24-206$) of the action potential (dV/dt) in volts per second (V/s) of surface fibres in the extensor digitorum muscle of the mouse as a function of days after nerve crush (filled circles) or section (squares) of the deep nerve. The figure is divided into two parts: (a) rate of rise in normal Krebs-Ringer solution (N.R.) and (b) rate of rise in Krebs-Ringer solution containing 10^{-6} M tetrodotoxin (N.R. 10^{-6} M TTX). The figures within parentheses indicate the percentage of fibres ($N = 31-103$) responding to the action potentials in the presence of TTX and the open circles indicate the rate of rise of normally innervated

values of the sarcolemma. These changes were of similar magnitude and time-course regardless of whether denervation was accomplished by nerve crush or section. Complete denervation was indicated at 3 and 6 days after surgery by the lack of a twitch after nerve stimulation and the absence of miniature end-plate potentials (m.e.p.p.s) and normal muscle fibres. The resting membrane potential (E_m) was reduced from a control (intact) value of -75.8 ± 0.4 mV to -56.6 ± 1.1 mV and -55.9 ± 1.5 mV at 6 days after nerve crush and section, respectively (Fig. 1). Denervation also caused alterations in some properties of muscle

fibre action potentials (Fig. 1). For example 6 days following nerve crush or section the rate of rise (dV/dt) of the action potential decreased in both the nerve-crushed (596 ± 15 V/s) and the nerve-sectioned (574 ± 4 V/s) muscles from an initial innervated value of 758 ± 14 V/s. Similarly the threshold potential for action potential generation increased from -41.5 ± 0.9 mV to -47.2 ± 0.9 mV in denervated and to -45.3 ± 0.8 mV in nerve-crushed muscles (9 day values).

At 3 days after denervation 70-90% of the muscle fibres examined had developed resistance to the blocking action of tetrodotoxin (TTX) and by 6 days all the fibres. The dV/dt of the TTX-resistant action potentials increased to peak values reaching 206 ± 13 V/s (nerve crush) and 273 ± 13 V/s (nerve section) at 6 days after denervation (Fig. 2).

Reinnervation was apparent in the nerve-crushed EDL muscles at 9 days after denervation with the appearance of m.e.p.p.s in individual muscle fibres along with weak muscle twitches after nerve stimulation. M.e.p.p.s and muscle twitches were not observed in any of the nerve-sectioned muscles during the entire time-course of the study. With the onset of reinnervation, there was a slight increase in the E_m for the nerve-crushed muscle (-63.4 ± 0.7 mV) compared to the nerve-sectioned muscle (-59.8 ± 0.9 mV). By the 12th day after nerve crush the E_m of the reinnervating EDL had increased substantially to -67.7 ± 0.8 mV while the nerve-sectioned muscle remained at -55.3 ± 0.9 mV. After this time the E_m of the reinnervating EDL increased at a slow rate and was near that for normally innervated muscle at 21 days after nerve crush (Fig. 1).

The recovery of the muscle action potential during reinnervation can be divided into two events: the recovery of action potential properties *per se* (e.g. dV/dt) and the recovery of muscle fibre sensitivity to the blocking action of TTX. Between 9 and 12 days after nerve crush the reinnervating EDL became rapidly sensitive to TTX. This is indicated by the reduction in the number of fibres with TTX-resistant action potentials from 87% at 9 days to 26% at day 12 and by a reduction in the dV/dt of the fibres with TTX-resistant spikes (Fig. 2). The dV/dt of the action potential in TTX-containing solution decreased from 206 ± 13 V/s at 6 days after nerve crush to 16 ± 3 V/s at 12 days. In contrast to the TTX-sensitivity the dV/dt of the action potential in normal Krebs-Ringer solution stayed at values similar to those in denervated fibres at 14 days

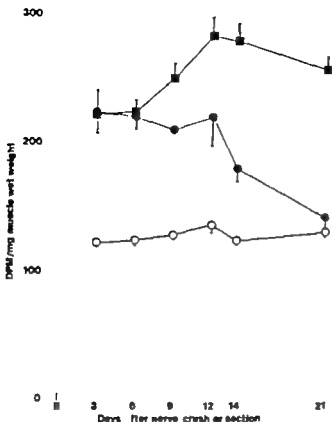


Fig. 3 The uptake (mean \pm S.E.) of 3 H inulin in disintegrations per minute per milligram muscle wet weight (DPM/mg muscle wet weight) by the extensor digitorum longus muscle of the mouse as a function of days after crush (filled circles, $N=8$) or section (squares, $N=8$) of the deep peroneal nerve. The open circles indicate the uptake of normally innervated muscle ($N=16$).

after nerve crush (567 ± 17 V/s) and did not approach values of innervated muscles (758 ± 14 V/s) until 21 days after nerve crush (705 ± 18 V/s) (Fig. 7). The recoveries towards values of innervated muscles of threshold potential and overshoot of the action potential during reinnervation occurred between 14–21 days.

Uptake of 3 H inulin. The endocytic activity of EDL muscles after denervation and during reinnervation as measured by the uptake of 3 H-inulin is shown in Fig. 3. Denervation by both nerve crush or section produced almost a 100% increase in the uptake per mg wet muscle weight at 3 days after denervation. The nerve sectioned muscles showed a further increase in uptake between days 6 and 12 followed by a plateau at about 150% of control values by 21 days. In contrast the nerve-crushed muscles showed no increase between days 6 and 12 and then slowly decreased their uptake after 1

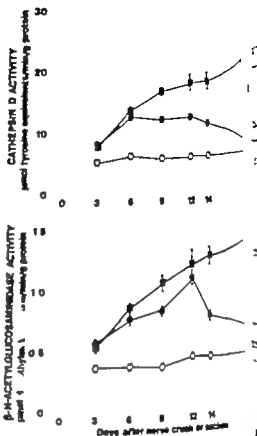


Fig. 4 The activities (mean \pm S.E.) of the enzymes cathepsin D (upper panel) expressed as tyrosine equivalents liberated/min/mg protein and N-acetylglucosaminidase (lower panel) expressed as 4-methylumbelliferone liberated/min/mg protein in innervated control muscles (open circles), nerve-crushed muscles (filled circles) and chronically denervated muscles (squares, $N=8$) as a function of days after nerve crush or section.

days reaching values similar to normally innervated muscles by 21 days.

Lysosomal enzyme activities. Denervation either nerve crush or section produced increases in the activities of the muscle lysosomal enzymes N-acetylglucosaminidase (NAG) and cathepsin D (Fig. 4). The activities of these enzymes in the nerve sectioned muscles increased steadily reaching apparent plateau values at 21 days. However the muscles whose nerve had been crushed showed a slower or no increase in activities between 6 and 12 days and decreased thereafter. By 21 days after nerve crush, the activities of NAG and cathepsin D were at or below innervated values while the nerve sectioned muscles showed 3–4 times higher activities.

SION

that some denervation-induced alterations in electrical properties are dependent on synthesized proteins (Gramp et al 1972) and that these proteins are incorporated into the cell membrane. It is likely that this type of turnover may be explained by the presence of endo- and exocytosis. The increase in the uptake of ^3H -ouabain in denervated skeletal muscle in the present study was similar to that reported previously for uptake of extracellular marker molecules after denervation by the vesiculation and internalization of surface membrane primarily in the tubular region (Libelius et al 1978; Libelius & Lundquist 1979). Since in the present study there was no change in muscle weight relative to control muscle at 3 days after nerve section, increased uptake per mg muscle clearly indicated an increase in endocytosis. Within that period the alterations in membrane electrical properties of denervation occurred. The further increase in uptake per mg muscle between 6 and 21 days after nerve section, was complicated by a concomitant loss in muscle weight, which was probably due to a decrease in fibre volume rather than number (Pellegrino & Franzini 1963). When the data were plotted as total DPM/muscle (not shown) against days after denervation, the uptake of the nerve-section muscles remained at a high, but constant, level between 3 and 12 days and thereafter displayed an apparent decrease at a slow rate. Therefore denervation induced high rates of endocytosis which was maintained at least 12 days after nerve section. No major changes in the membrane electrical properties were observed through 21 days after nerve section beyond the initial denervation-induced alterations, which occurred within the first 6 days.

In the nerve-crushed muscles, the uptake of ^3H -ouabain per mg muscle weight after denervation increased in a fashion similar to the nerve-section muscles. While the uptake per mg muscle increased between 6 and 12 days in the nerve-sectioned muscles, the uptake per mg muscle in the nerve-crushed muscles remained constant during this same period. In both the muscles in both preparations decreased in weight during this time. It may be concluded that the processes which were reinnervated decreased their endocytic activity. This decrease occurred between

6 and 9 days after nerve crush. At times later than 12 days after nerve crush the decrease in uptake per mg muscle resulted from the combined effects of a decrease in total uptake per muscle and an increase in muscle weight. It is thus apparent that the increased endocytic activity initiated by denervation is reversed by reinnervation.

Reinnervation occurs between 6 and 9 days after nerve crush because miniature endplate potentials were present at 9 days. The results demonstrated that recovery of some membrane electrical properties during reinnervation were not temporally related. That is, the time-course of recovery differed between individual electrical properties e.g. E_m , rate of rise (dV/dt) of the muscle fibre action potential and TTX sensitivity. The repolarization of the membrane during reinnervation was similar to that previously reported (McArdle & Albuquerque 1973). The present result suggests that the increase of the E_m occurs in two stages: a rapid increase at the onset of reinnervation between 6 and 12 days, and a slower increase between 12 and 21 days. In addition dV/dt and TTX-sensitivity did not recover at the same rate as shown previously in a preliminary report (Sjölin & McArdle 1977). By 12 days after nerve crush the reinnervated muscles, like innervated muscle were almost completely sensitive to the blocking action of TTX. In contrast the dV/dt in the absence of TTX remained at a value similar to chronically denervated muscle through 14 days after nerve crush and only approached innervated values at 21 days.

The difference in recovery rates between and within the above parameters may be interpreted in the following manner. The changes in membrane electrical properties occurring during reinnervation depend on the morphology and lipid composition of the membrane in addition to the removal and insertion of specific membrane proteins. It appears likely that the early rapid repolarization of the membrane and the early abolishment of TTX-sensitivity are related to the removal of proteinaceous material responsible for sodium permeability in the muscle membrane. In regard to E_m it is believed that the depolarization caused by denervation is, at least in part, secondary to an increase in the sodium leak permeability of the muscle membrane (Robbins 1977). TTX-insensitive channels are presumably voltage-sensitive sodium channels. The late slower repolarization could result from conductance changes which similar to dV/dt may depend on the

relationship of ion channels to their environment. It is interesting to note that the decrease in membrane resistance which reflects an increase in ionic conductances occurs at the later stages of reinnervation after an early increase in E_m (McArdle & Albuquerque 1973).

Earlier studies suggested that increased endocytosis in denervated skeletal muscle may activate lysosomal mechanisms thereby fostering degradative processes (Libelius et al. 1978). The present study provides further evidence for at least a temporal relationship between endocytosis and the activity of the lysosomal system. The activities of the proteolytic acid hydrolase cathepsin D and the acid glycosidase N-acetylglucosaminidase (NAGA) increased steadily after nerve section while leveling off with the onset of reinnervation and then decreased to near innervated values by 21 days after nerve crush in conformity with changes in endocytic activity and membrane electrical properties. The exact mechanisms by which the lysosomal system is implicated in degeneration and recovery of the muscle in denervation reinnervation remain to be elucidated. It seems likely however that the activation of the lysosomal system by endocytosis is responsible for the breakdown and/or recycling of membrane constituents.

The study was supported by grants from the Medical Faculty, University of Lund, Sweden and the Swedish Medical Research Council, Stockholm, Sweden (14X-311-04X-4286) and from the Muscular Dystrophy Association. L.C.S. was supported by a postdoctoral fellowship from the National Institute of Neurological and Communicable Diseases and Stroke, USA.

REFERENCES

- ALBUQUERQUE E. X. & THESLEFF S. 1968. A comparative study of membrane properties of innervated and chronically denervated fast and slow skeletal muscle of the rat. *Acta Physiol Scand* 73: 471-480.
- BARRETT A. J. 1972. Lysosomal enzymes. In: *Lysosomes: a laboratory handbook* (ed. J. T. Dingle) pp. 46-135. North-Holland Publishing Company, Amsterdam.
- GRAMPP W., HARRIS J. B. & THESLEFF S. 1972. Inhibition of denervation changes in skeletal muscle by blockers of protein synthesis. *J Physiol* 221: 743-754.
- KATZ, B. & THESLEFF S. 1957. On the determination of the amplitude of the membrane potential. *J Physiol* 137: 767-778.
- LIBELIUS R., JOSEFSSON J. O. & LUNDQVIST I. 1979. Endocytosis in chronically denervated skeletal muscle: a biochemical and ultrastructural study with horseradish peroxidase. *Ann NY Acad Sci* 283: 792.
- LIBELIUS R., LUNDQVIST I. & THESLEFF S. 1978. Intracellular uptake and degradation of extracellular tracers in mouse skeletal muscle: in vitro the effect of denervation. *Ann NY Acad Sci* 304: 641-647.
- LOWRY O. H., ROSEBROUGH N. J., FARR A. L. & RANDALL R. J. 1951. Protein measurement with Folin phenol reagent. *J Biol Chem* 193: 265-275.
- McARDLE, J. J. & ALBUQUERQUE E. X. 1973. A study of reinnervation of fast and slow muscle. *J Gen Physiol* 61: 1-13.
- PELLEGRINO C. & FRANZINI C. 1963. An electron microscope study of denervation atrophy in white skeletal muscle fibers. *J Cell Biol* 17: 123.
- REDFERN P. & THESLEFF S. 1971a. Acetylcholine generation in denervated rat skeletal muscle. I. Quantitative aspects. *Acta Physiol Scand* 85: 564.
- REDFERN P. & THESLEFF S. 1971b. Acetylcholine generation in denervated rat skeletal muscle. II. The action of tetrodotoxin. *Acta Physiol Scand* 82: 70-78.
- ROBBINS N. 1977. Cation movements in short-term denervated rat fast-twitch muscle. *J Physiol* 271: 605-624.
- SELLIN L. C. & McARDLE J. J. 1977. Recovery of the characteristics of skeletal membrane potentials during reinnervation. *The Neurologist* 19: No. 395.
- SELLIN L. C. & THESLEFF S. 1979. Membrane electrical properties during long-term denervation of rat skeletal muscle. *Acta Physiol Scand* (in press).
- SELLIN L. C., LIBELIUS R. & TÄGERFLO M. 1979. Membrane alterations in denervation and during reinnervation of mouse skeletal muscle. *Acta Physiol Scand Suppl* 471.
- THESLEFF S. 1966. Spontaneous electrical activity in denervated rat skeletal muscle. In: *The effect and release on neuromuscular function* (ed. E. Mann and P. H. H. H.) pp. 41-51. Czechoslovak Science Press, Prague.
- WEINSTOCK I. M. & IODICE, A. A. 1969. Adenosine triphosphatase activity in muscular dystrophy and denervation. In: *Lysosomes in biology and pathology* (J. T. Dingle and H. B. Fell) vol. 1 pp. 49-64. North-Holland, Amsterdam.

Morphological and histochemical properties of tongue muscles in cat

E. HELLSTRAND

Dept of Physiology, Karolinska Institutet, Stockholm, Sweden

HELLSTRAND E. Morphological and histochemical properties of tongue muscles in cat. *Acta Physiol Scand* 1980; 110: 187-198. Received 4 Jan 1980. ISSN 0001-6772. Department of Physiology, Karolinska Institutet, Stockholm, Sweden.

The morphology of the tongue muscles was studied by *in situ* dissection as well as by histological and histochemical methods. By means of the latter an anatomical rearrangement of attachment and fiber courses was made. The histochemistry was studied in sections stained for myofibrillar adenosine triphosphatase (mATPase), succinic dehydrogenase, NADH dehydrogenase, phosphorylase, esterase, glycogen and lipids. Fibers of type I and type II were identified, and the latter were subdivided into II₁ (highly glycolytic), II₂ (intermediately glycolytic and lipolytic) and II₃ (highly lipolytic). In the *striated* muscles, the fibers were 19-25% type I (mean diameter 77 µm) and 75-81% type II (37 µm); the three type II subgroups appeared in equal proportions, each accounting for 22-30% of the total fiber amount. *Pars longitudinalis superior* m. hyoglossi and *pars longitudinalis inferior* m. styloglossi contained only type II fibers, mainly type II₁ (67% and 46% respectively), of diameters like those in m. hyoglossus and m. styloglossus. The *intrinsic* muscles also consisted entirely of type II fibers (23 µm). II₃ fibers predominated in m. verticalis (83%), which has only 10% I and 6% II, whereas the fiber composition of m. transversus was more balanced: 37% type I, 32% II₁ and 31% II₃.

Key words: Tongue muscles, cats, anatomy, histochemistry, fiber classification.

of the cat, with mainly intraoral action (classification (the type I category according to & Baggett 1971) is provided with muscles of extrinsic or intrinsic according to whether they partly or totally within the tongue. Functionally the extrinsic muscles are usually classified as protruders or retractors and the intrinsic as shape-modelling agents.

Morphologically the muscles of the tongue have been studied extensively (e.g. Ludwig Ferdinand Edgeworth 1935, Abd-El-Malek 1938, Dabbs 1951, Reighard & Jennings 1961, Doran 1975). The resulting reports often differed about the of insertion and fiber courses of individual muscles. Precisely which muscles contribute to the movement of the tongue has therefore been debated.

However, the histochemical techniques mostly used to examine enzyme activities and metabolite contents within muscle fibers can be combined with conventional histology and in this way, so that the course of fibers from indi-

vidual muscles can be determined more reliably than before. The present study of morphological and histochemical properties of the tongue muscles employed such an approach. The results are intended to serve as basis for further studies on the functional properties of these muscles.

MATERIAL AND METHODS

Basic information on tongue muscle morphology was obtained from textbooks (e.g. Reighard & Jennings 1961) and evaluated through *in situ* dissections of 8 cats under Zeiss dissection microscope. Some of the material dissected had been fixed in 10% formal. The general view of the muscle topography thus obtained guided the subsequent histological and histochemical studies.

Histological procedure. Tongues from 5 adult cats (2.3-4.6 kg) were dissected along the septum mediantum, fixed in 10% formal and embedded in paraffin. Frontal sagittal and transverse sections were cut and stained with (1) iron-haematoxylin and eosin and (2) Weigert-van Gieson solution. For further details on the staining procedures see Roman (1968).

Histochemical procedure. Biopsies were done on the tongue muscles of 11 adult cats (2.5-4.5 kg) and the

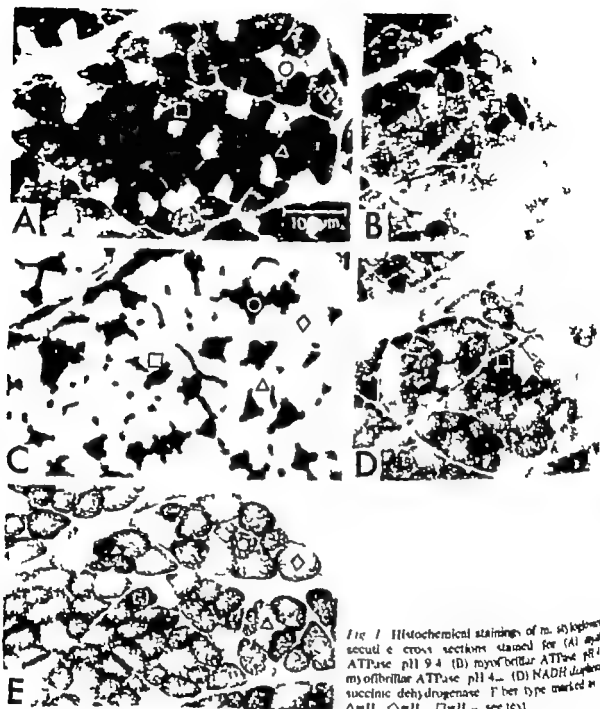


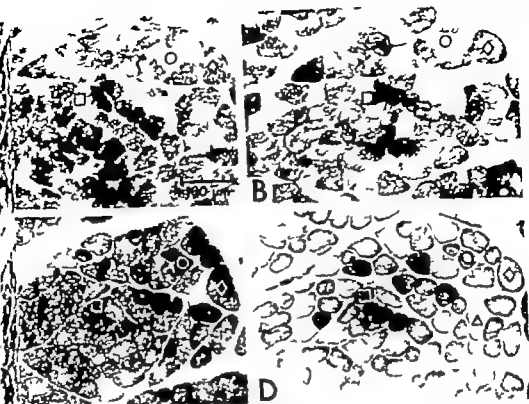
Fig. 1. Histochemical stainings of *m. styloglossus* secuted cross sections stained for (A) myofibrillar ATPase pH 9.4 (B) myofibrillar ATPase pH 4.0 (C) NADH diaphorase (D) succinic dehydrogenase. Fiber type marked as $\Delta=II$ $\diamond=II_m$ $\square=II_n$ see text.

specimens were immediately frozen in isopentane previously cooled in liquid nitrogen. Sections 1–16 μm thick were cut on a microtome at -20°C and stained.

For the parts of the extrinsic muscles running outside the tongue mass biopsy material was taken from the origin of the muscles up to the middle part of the bellies (i.e. where the muscles pass into the tongue). For biopsy of the parts of the extrinsic muscles running within the tongue and for the intrinsic muscle cubes (side 15 mm) were cut from the anterior middle and posterior parts of the tongue. All samples were sectioned on the microtome in three perpendicular planes, so as to expose the muscles in cross section (e.g. in the frontal plane para lon-

gitudinally superior = hyoglossal and para longitudo inferior = styloglossal in the sagittal plane in transverse and in the transverse plane in vertical).

Stainings: (1) myofibrillar ATPase (Padykula & Hensen 1957) after preincubation in sodium barbital buffer at pH 11 in barbital acetate buffer at pH 4.6 and 4.2 (Davies & Brooke 1973); (2) succinic dehydrogenase (Nadler 1957); (3) NADH diaphorase (Searle, Hens & Padykula 1957); (4) phosphorylase α (Takeuchi & Kuriaki 1957); (5) glycogen (McCarthy & Bitter 1973); (6) esterase (Hens & Winters 1973). Lipid (Carlsson & Drury 1957) light modifications



Histochromal stainings of an styloglossus. Consecutive cross sections stained for (A) phosphorylase, (B) succinate dehydrogenase, (C) lipids, (D) esterase. Fiber types marked as in Fig. 1.

carrying in Nyström (1968), for further details see (1968).

Enzyme and density measurements. The stained tissues are examined in slides under the microscope in micrographs.

In histochemically stained fibers the relative density measured from pseudochromatic black and white pictures (Pantascopic X Kodak) by means of an integrating microdensitometer (Model MX III C, Luch & C Ltd), each operates on double light system connected to photomultiplier and density directly and linearly. Baseline as set in unexposed part of each negative.

Enzyme reactions. As representative example of the activity and substrate content of the muscles are shown in Fig. 1A-E and Fig. 2A-D (all are cross sections). The following presentation of staining reactions shows also brackets the mean activity or ranges of density of the fibers stained.

Fibrillar ATP. After preincubation and staining at pH 9.4, fiber types could be distinguished: type I with low enzyme activity (0.65), and type II with high activity (1.45-1.58). Preincubation at pH 4.2 showed the type I high (1.28-1.00) and type II low (0.11). Preincubation at pH 4.4, three levels of activity were

seen: high (1.33), intermediate (0.95) and low (0.78). Type I fibers showed only high enzyme activity whereas type II fibers exhibited all three levels. See Fig. 1A, B and C for the staining reactions at pH 9.4, 4.8 and 4.2 respectively.

Succinate dehydrogenase and NADH dehydrogenase. Fig. 1E shows the staining reactions for the succinate dehydrogenase. The distribution of the formazan granules within the fibers varied, disclosing 3 categories of fibers: those with largely diffuse distribution of granules, those with diffuse granules plus clear subsarcolemmal formazan activity and those showing anterior streak of formazan together with the subsarcolemmal and diffuse granules. Every type I fiber belonged to the third group while type II fibers were found in all three.

The staining pattern of NADH dehydrogenase (Fig. 1D) was essentially the same, although the granules were denser and the anterior streaks more pronounced than with the dehydrogenase staining. This might reflect the different distributions of these two enzymes within the muscle cells, since succinate dehydrogenase occurs only mitochondrially whereas NADH dehydrogenase is found microsomeally as well (de Duve, Wattiaux & Sanderson 1966). The type I and II fibers were grouped as for the dehydrogenase.

Phosphorylase and glycogen. The activity of phosphorylase (active phosphorylase see Chayes, Bittensky

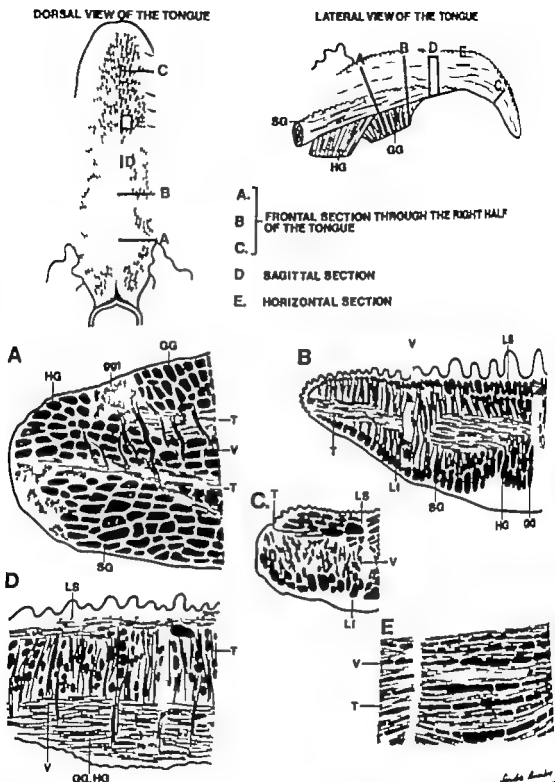


Fig. 3. The cat's tongue. Dorsal and lateral views of the tongue illustrated together with frontal (A, B, C), sagittal (D) and horizontal (E) sections through the right half of the tongue. GC = genioglossus; HG = hyoglossus; SQ = styloglossus; LS = pars longitudinalis superior in hyoglossus; LI = pars longitudinalis inferior in styloglossus; V = verticalis; gl = glandulae linguales.

& Butcher 1973). In the muscle fibers is indicated by Fig. 2A. Type I fibers had low activity (0.70) and type II intermediate (1.04–0.93) to high (1.18).

The staining for glycogen content (Fig. 2B) was less darkly (0.60) than type II fibers (1.1). The reaction like that for ph.

were high in phosphorylase proved low in. This glycogen depletion more likely arose from chemical procedures than from exhausting work, work also depletes the amount of active phosphate (Cori 1945; Kupeberg & Edstrom 1968).

Lipids. The type I fibers showed only high activity (1.08); type II had high (1.08), intermediate (0.94) and low (0.48) levels (Fig. 2D).

Lipid levels were related to the lipid content of fibers high in esterase activity storing more lipids than low enzyme activity. Type I fibers store lipids (1.43); type II are highly (1.38), intermediate (1.32) or highly (0.83) stained.

Fiber size. The fiber sizes in the tongue muscles were determined from micrographs of cross sections stained for lipid (Fig. 4). Fiber diameter (Dobowitz & 1973) as the index of fiber size (see also Brooke 1969).

Statistical method. Different muscles or fiber groups compared with Student's *t*-test. The differences were not significant ($P < 0.01$) (see also Bailey 1979).

TS

logy

Tongue muscle morphology was studied by dissection and by histological and histochemical analyses of biopsy materials. Data on each are presented below together with remarks for investigations. To begin with general morphology, Fig. 3 offers dorsal and lateral views of the tongue as well as drawings of frontal sagittal and horizontal sections through the right half of the tongue.

The dorsal view of the tongue in Fig. 3 shows the surface with the sites of the frontal (A, B, C), (D) and horizontal (E) sections indicated.

The papillae, plica glossoepiglottica mediana and central fold rising from the dorsal tongue surface to the epiglottis and the cranial surface of the epiglottis are sketched.

The lateral view of the tongue includes sites of sections (A-E), and topography of the extrinsic muscles, with the ventrolateral part of the tongue from its mucous membrane to clarify the relationship among the individual muscles.

The frontal section through the right half of the tongue demonstrates the posterior (A), middle (B) and anterior (C) parts. The drawings were based on micrographs of histochemically stained cross sections and retain the true relative proportions. In A, the genioglossus occupies the ventral part, m. genioglossus in the dorsomedial and medial parts and m. hyoglossus in the area between these two muscles, its

fascicles being distributed obliquely to the vertical plane.

In B the bundles of m. genioglossus and m. hyoglossus, comprising both type I and type II fibers (see Histochemistry below) run medial to the mixed fiber portion of m. styloglossus. Lateral to the mixed fiber portion of m. styloglossus, lateral to the pars longitudinalis inferior m. styloglossus (type II fibers only) forms a thin layer that narrows further as it nears the side of the tongue. In order to exemplify origins and insertion areas of m. transversus and m. verticalis in the same picture both muscles are shown together although in view of their topography (see below) only one muscle should be seen.

In C the fibers operating the tongue tip (all type II) are shown. Although small fiber bundles of the transverse and vertical muscles are present fibers of the longitudinal muscles predominate.

The sagittal section (D) shows the muscle topography half way along the tongue near the midline. The drawing was made from sections stained histochemically a procedure that usually deforms muscle fibers more than the histochemical treatment.

The horizontal section (E) of the medial part of the tongue's right half was cut just below the superior longitudinal fibers. It reveals the topography of m. transversus and m. verticalis (specimens histochemically stained).

Protruding the tongue

M. genioglossus. Its fibers were found to originate from the tubercle close to the symphysis of the lower jaw dorsomedial to the origin of m. genioglossus. The muscle passes within the lingual frenulum into the ventral part of the medial aspect of the tongue. The fibers then fan out in the vertical plane forming a thin muscle sheet which is inserted into the submucosal layer immediately below the superior longitudinal fibers of m. hyoglossus (see below). The insertion area extends from the anterior to the posterior part of the tongue. Posteriorly the muscle forms the medial part of the tongue root, together with m. hyoglossus. Anteriorly the fibers run more vertically to reach their insertion points terminating at least 1.5–2 cm from the tip's edge. The anterior portion of the muscle relates lateral to the fascicles of m. styloglossus.

According to e.g. Abd-El-Malek (1938) and Dabelow (1951) the genioglossal muscle also inserts into the hyoid bone, the epiglottis and the tongue tip. No fibers could be traced to these structures in

this study however. The present findings concur with those of Doran & Baggett (1977) who denied that the anterior reach of the muscle extends to the tip.

The two genioglossal muscles of the tongue were strictly separate. None of the fibers from one intermingled dorsally with those from the opposite side (cf Dabelow 1951; Reighard & Jennings 1961).

Retracting the tongue

1 *M. hyoglossus*. The muscle arose from the body of the hyoid bone and from the proximal part of its cranial cornu (i.e. the lesser horn of human anatomy according to Reighard & Jennings 1961). It runs parallel to the midline of the neck, making up the middle part of the tongue root, with *m. genioglossus* lying medial and *m. styloglossus* lateral to it. Most of the fibers spread obliquely fanlike in the vertical plane parallel to *m. genioglossus*. They insert into the dorsal submucosa from its posterior to its middle portion. Some fibers in contrast unite and run parallel to the dorsal tongue surface to insert into the tongue tip, forming its dorsal muscle layer and acting as the tip dorsoflexor. These fibers—previously termed *m. longitudinalis superior*—appear as a thin transverse sheet under the dorsal tongue epithelium. In this study the muscle sheet is named *pars longitudinalis superior m. hyoglossi* (see Histochemistry below). The hyoglossal muscle is paired.

The origin of *m. hyoglossus* has been variously attributed to the caudal cornu of the hyoid bone (greater horn Braus 1974; Cunningham 1964; Gray 1973), its cranial cornu (Abd El-Malek 1938) and its body (Reighard & Jennings 1961). This study found no fibers originating from the caudal cornu. The inserting fibers have been said to intermingle with fibers of *m. longitudinalis inferior* and *m. styloglossus* (e.g. Cunningham 1964) but both of the hyoglossal muscle portions identified here were well separated from the other tongue muscles (see also Dabelow 1951).

2 *M. arylglossus*. The muscle originates from the cranial cornu of the hyoid bone from the area around the tympanic bulla and from the styloid process. Its fibers enter the lateral part of the posterior tongue forming the lateral part of its root, with *m. hyoglossus* lying both dorsal and medial to it and *m. genioglossus* medially. It runs parallel to the ventral epithelium in a triangular shape that shrinks anteriorly. The main fiber portion lies in the middle of

the forward half of the tongue. A *septa* enters the tongue tip to form its ventral wall and act as the tip ventroflexor. This is here called *pars longitudinalis inferior m. glossi* (see Histochemistry).

M. styloglossus has a rather everted origin compared to the genioglossal and hyoglossal muscles. Its two fiber portions were very fine, having no fibers mixed with *m. larynx* (cf Cunningham 1964) or *m. geniohyoid* (below 1951). Abd El-Malek's (1938) septum (Dabelow 1951) Abd El-Malek's (1938) septum the muscle winds around the lateral and parts of the inferior longitudinal *septa* before the middle of the tongue posteriorly was not borne out. On the contrary, the *m. styloglossus* muscle run in close formation, only the medial one third of the tongue free, other two extrinsic muscles. According to D. (1951) after entering the tongue root a bundle of *m. styloglossus* runs to the opposite side of the tongue on its way mixing with fibers of *m. larynx* versus to end up attached to the styloid process the opposite side. It thus constitutes a connection between the two styloid processes (also Hesse 1875). This was not confirmed in present study.

Shaping the tongue

1 *M. transversus*. The muscle consists of very finely running fibers originating from the septum of the tongue and from the fibers surrounding *m. genioglossus* and *m. larynx*. The fibers form thin sheets paralleling one another in the frontal plane and inserting into the integument of the tongue. The muscle fills the central part of the tongue but becomes narrower toward the root and tip end. Some of the fibers are inserted between fascicles of *pars longitudinalis superior m. glossi*. Ventrally it runs above *m. styloglossus* with some fibers inserted among the fascicles of *pars longitudinalis inferior m. styloglossus*. As no fibers of the left or the right transverse muscles are seen to cross the midline of the tongue not even where the median septum is present the muscle is considered paired (cf Braus 1974; Dabelow 1951). Its close connection to the median septum is well established (e.g. Hesse 1875; Wig Ferdinand 1884; & Markowski).

951). No sign of the reported connection transverse fibers and the origin of *m. us* (Dabelow 1951) was found.

sheet arrangement of the transverse sheets is easily demonstrated by sagittal and l sectioning. A plating of transverse and fibers as described by previous investigators not seen except at the far edge of the among the inserting fibers.

verticalis The muscle originates from al submucosa under pars longitudinalis *m. hyoglossi*, minor portions also come sheath around fascicles of this muscle. It sets which are organized in parallel in the lant. The vertically running fibers are m- to the ventral submucosa or among the of the muscles in the ventral muscle layer id

pography of the muscle is clearly evident al and sagittal sections. Its extension is that of *m. transversus* their sheets of crasing.

longitudinalis superior and m. lon- of nor Previous studies considered scales intrinsic with well-defined oner courses and insertions. *M. longitudinalis* was said to originate from the os *m. membrana hyoglossi* and the dorsal membrane, its fibers forming the dorsal layer and being inserted into the tongue tip. *longitudinalis inferior* was said to extend from hyoiden and epiglottis to the tongue tip posteriorly between *m. genioglossus* (me and *m. hyoglossus* laterally), then anteriorly *m. m. genioglossus* and *m. styloglossus*, lose *m. m. genioglossus* and the lingual vessels

present study defines both muscles different- superior longitudinal muscle was shown to ber portion of the hyoglossal muscle. The longitudinal muscle was found to extend ie tongue tip to the origin of the styloglossal running in the lateral part of the tongue, to *m. hyoglossus*. Hence Dabelow's (1951) that short, longitudinal superficially num- bers belong to proper intrinsic muscle, al only the longer more deeply located fibers ie from extrinsic muscles was disproved as existence of *m. longitudinalis proprius* s. (Ludwig Ferdinand 1884; Nussbaum & Mar # 1896) borne out.

er minor *M. genioglossus* consists of about

1 600 fibers, *m. hyoglossus* and *m. styloglossus* con- tam around 2000 each. The number of fibers form- ing a single muscle sheet (around 150 μ m thick) of *m. transversus* or *m. verticalis* varies widely the maximum (at the central part of the tongue) is around 1 300-1 400. Pars longitudinalis superior *m. hyoglossi* consists of about 700 fibers in the anterior part of the tongue where pars longitudinalis inferior has somewhat fewer around 600 only about one fourth of these anterior fibers reaches the dorsal and ventral tip.

Histochemistry

Muscle fibers that had similar histochemical stain- ing reactions were grouped then classified as type I or type II on the basis of their staining reactions for myofibrillar ATPase pH 9.4 (terminology of Fenebel & Engel 1963). An index number (1-3) added to the type symbol designated each group's staining reaction to succinic dehydrogenase: 1 dif- fuse spread of formazan granules, 2 distinct sub- sarcolemmal activity of formazan, 3 distinct in- terior streaks of formazan.

Table 1 summarizes the histochemical profiles of the groups. The *I₁₁₁* fiber appears to possess a high oxidative capacity together with high I polytic and low glycolytic capacities. The reverse applies to the II. The *II₁₁₁* fiber is intermediate in all three re- spects and the *II₁₁₁* group is uniformly high.

One fiber from each group is labelled in Fig. 1 and to illustrate their histochemical profiles.

Fiber composition and size in extrinsic muscles

All 4 fiber groups are represented in these muscles with the type II varieties (*II₁₁₁*, *II₁₁₁*) clearly pre- dominant. The mean diameter for type II fibers is around 36 μ m and the mean for type I about 28 μ m. The fibers designated II and *II₁₁₁* have similar diameters and are significantly larger (by about 10 μ m) than the *II₁₁₁* group. The frequencies and sizes of type I and type II fibers are listed in Table 2.

II genioglossus (Data collected from the part running outside the tongue mass.) No fiber group preponderates each having about 25% frequency of occurrence. Cross section of the muscle showed no clustering of fibers of any group only irregular scattering.

The mean diameter of the *II₁₁₁* group is small, around 27 μ m, and its fibers are among the finest in the extrinsic muscles. The mean diameters of the II

this study however. The present findings concur with those of Duran & Baggett (1977) who denied that the anterior reach of the muscle extends to the tip.

The two genioglossal muscles of the tongue were strictly separate. None of the fibers from one intermingled dorsally with those from the opposite side (cf. Dabelow 1951; Reighard & Jennings 1961).

Retracting the tongue

1 *M. hyoglossus*. The muscle arose from the body of the hyoid bone and from the proximal part of its cranial cornu (i.e. the lesser horn of human anatomy according to Reighard & Jennings 1961). It runs parallel to the midline of the neck making up the middle part of the tongue root with *m. genioglossus* lying medial and *m. styloglossus* lateral to it. Most of the fibers spread obliquely fanlike in the vertical plane parallel to *m. genioglossus*. They insert into the dorsal submucosa from its posterior to its middle portion. Some fibers in contrast unite and run parallel to the dorsal tongue surface to insert into the tongue tip forming its dorsal muscle layer and acting as the tip dorsoflexor. Those fibers—previously termed *m. longitudinalis superior*—appear as a thin transverse sheet under the dorsal tongue epithelium. In this study the muscle sheet is named *pars longitudinalis superior m. hyoglossi* (see Histochemistry below). The hyoglossal muscle is paired.

The origin of *m. hyoglossus* has been variously attributed to the caudal cornu of the hyoid bone (greater horn Braus 194; Cunningham 1964; Gray 1973), its cranial cornu (Abd El Malek 1938) and its body (Reighard & Jennings 1961). This study found no fibers originating from the caudal cornu. The inserting fibers have been said to intermingle with fibers of *m. longitudinalis inferior* and *m. styloglossus* (e.g. Cunningham 1964) but both of the hyoglossal muscle portions identified here were well separated from the other tongue muscles (see also Dabelow 1951).

2 *M. styloglossus*. The muscle originates from the cranial cornu of the hyoid bone from the area around the tympanic bulla and from the styloid process. Its fibers enter the lateral part of the posterior tongue forming the lateral part of its root with *m. hyoglossus* lying both dorsal and medial to it and *m. genioglossus* medially. It runs parallel to the ventral epithelium in a triangular shape that shrinks anteriorly. The main fiber portion lies in the middle of

the forward half of the tongue. A single fiber enters the tongue tip to form its ventral muscle and act as the tip ventroflexor. This fiber is here called *pars longitudinalis inferior m. glossi* (see Histochemistry).

M. styloglossus has a rather extensive origin compared to the *genioglossal* and *hyoglossal* muscles. Its two fiber portions were coarse having no fibers mixed with it here (cf. Cunningham 1964) or *m. genioglossus* below (1951). Abd El-Malek's (1938) and Dabelow's (1951) *Abd El-Malek's* (1938) report the muscle winds around the lateral and parts of the inferior longitudinal muscle before the middle of the tongue posteriorly was not borne out. On the contrary the fibers of *styloglossus* muscle run in close formation with only the medial one-third of the tongue root. The other two extrinsic muscles. According to Dabelow (1951) after entering the tongue root a sheet of *m. styloglossus* runs to the opposite side of the tongue on its way mixing with fibers of *m. genioglossus* to end up attached to the styloid process on the opposite side. It thus constitutes a connection between the two styloid processes (also Hesse 1875). This was not confirmed in the present study.

Shaping the tongue

1 *M. transversus*. The muscle consists of variously running fibers originating from the septum of the tongue and from the firm tissue surrounding *m. genioglossus* and *m. hyoglossus*. The fibers form thin sheets paralleling one another in the frontal plane and inserting into the integument of the tongue. The muscle thickens the central part of the tongue but becomes thinner toward the root and tip each. Some of the fibers are inserted between the fascicles of *pars longitudinalis superior m. glossi*. Ventrally it runs above *m. styloglossus* with some fibers inserted among the fascicles of *pars longitudinalis inferior m. styloglossi*.

As no fibers of the left or the right transverse muscles are seen to cross the midline of the tongue not even where the median septum is present the muscle is considered paired (cf. Braus & Dabelow 1951). Its close connection to the median septum is well established (e.g. Hesse 1875; wig Ferdinand 1884; Nussbaum & Markowicz

axons or fascicles of very fine fibers such as to create a place of attachment for the longibers. Rather the dorsal fibers spread as hyoglossal fibers in the root ending in the body of the hyoid bone. These fibers lie in the same plane as the fibers of m. 1. During their course from the origin they form distinct fascicles, easily identifiable in the middle of the dorsal tongue running up to the tip. The composition is mainly I (67%), the rest being equal parts II and III fiber types are irregularly scattered in the muscle portion.

It can be expected the fiber diameters of this deep hyoglossal muscle do not differ significantly from those measured in the undivided muscle in the tongue.

Longitudinalis inferior m. styloglossus: (Data collected from the part of the tongue mass.) The composition is 28% I (31 μ m) and 27% II (41 μ m), followed by fibers at 22% (39 μ m) and the II_{123} fibers at 1 μ m). Distributions of all types are irregular.

Longitudinalis superior m. hyoglossus: Section of the tongue as described for pars longibers superior m. hyoglossus revealed another portion made up exclusively of type II fibers. It is followed from the ventral part of the tip to the lateral parts of the ventral tongue to the root. Its fibers ceased to be separate in the medially lying styloglossus which consists of type I and type II fibers but spread among them. Thus, both fiber portions are inserted in the same points of origin with their fibers used.

Fiber composition of this ventrolateral myofascium is primarily II (46%) with 40% II_{123} and 15% type I. All are irregularly scattered. Diameters are about the same values found in the longitudinal part outside the tongue.

Extrinsic and accessory muscles

Fiber composition of these muscles is almost entirely type II very rarely a single type I fiber can be found among the fiber bundles. The mean diameters are about 23 μ m, making these the finest of the tongue. As with the extrinsic muscles, the II_{123} are the largest and the II_{123} the smallest.

The largest group is the I at

37% (mean diameter 30 μ m) followed by the II_{123} at 32% (26 μ m) and the II_{123} at 31% (20 μ m). Although all three kinds of fibers are mixed together in fascicles, the fascicles inserting into the most lateral part of the tongue seem to have more I fibers than the other fascicles.

M. verticalis: The II_{123} fiber group is conspicuous representing 83% of the total composition (mean diameter 20 μ m). The remainder consists of 10% I (28 μ m) and 6% II_{123} (14 μ m). The fascicles appear fairly homogeneous in cross section, the clusters of II_{123} fibers shot with fibers of the scarcer groups.

DISCUSSION

The tongue muscles attachments and fiber courses as reported here differ in part from earlier findings. It is conceivable that the sampling techniques employed by previous investigators obscured certain aspects of the tongue muscle anatomy or that species differences among the animals studied were not taken into account. In any event, previous histological methods of tissue preparation and staining certainly deformed the tissues more than modern histochemical procedures. Compare for example Fig 3 D and E, the intrinsic muscles drawn from histological sections (D) are much less distinct than those drawn from histochemical sections (E). Only the histochemical treatment reveals unequivocally the parallel alternation of m. transversus and m. verticalis in sheets.

The morphological findings presented here are compatible with the various functions of the muscles, such as tongue protrusion or retraction, tip flexion or changing the tongue's shape. If m. geniohyoid was attached to the hyoid bone—as stated by e.g. Abd. El-Malek 1938 but here disproved—this muscle would have to subserve jaw opening (cf. anatomy and function of m. geniohyoides according to Reighard & Jennings 1961). No such function has ever been ascribed to it (Edgeworth 1935; Lowe 1976). If the same muscle were attached to the tongue tip (e.g. Debelow 1951) it would have to be a tip ventroflexor—a role rejected by Doran & Baggett (1972) in their reassessment of the anatomy of m. geniohyoides. The present study found no such attachment, thus upholding their analysis and extending it to cat.

The longitudinally running muscle fibers are here defined as belonging to the retractors.

Table 1 *Histochemical fiber profiles of the tongue muscles*

Staining reactions with mean relative density or ranges of density shown within brackets

I ₁₂	II	II ₁	II ₂₂	Enzyme stain
Low (0.88)	High (1.45-1.28)	High (1.45-1.28)	High (1.45-1.28)	Myofibrils (2) pH 9.4
High (1.28-1.00)	Low (0.11)	Low (0.11)	Low (0.11)	Myofibrils (7) pH 4.2
High (1.3)	High (1.33)	Intermediate (0.95)	Low (0.78)	Myofibrils (7) pH 4.6
Diffuse sub-sarcolemmal in tenor streaks	Diffuse	Diffuse sub-sarcolemmal	Diffuse sub-sarcolemmal interior streaks	Succinic dehydrogenase (granular distribution)
Diffuse sub-sarcolemmal interior streaks	Diffuse	Diffuse sub-sarcolemmal	Diffuse sub-sarcolemmal interior streaks	NADH dehydrogenase (granular distribution)
Low (0.70)	High (1.18)	Intermediate (1.08)	High-intermediate (1.18-0.93)	Phosphorylase
Low (0.60)	High (1.1)	Intermediate (0.88)	High (0.98)	Glucopyruvate
High (1.08)	Low (0.48)	Intermediate (0.95)	High (1.08)	Esterase
High (1.43)	Low (0.85)	Intermediate (1.32)	High-intermediate (1.38-1.30)	Lipids

and II₁ fibers are 40 μ m and 39 μ m respectively the mean for II₂₂ fibers is about 30 μ m.

At hyoglossus (Data collected from the muscle part running outside the tongue mass.) At a frequency of around 19% group I₂₂ fibers (mean diameter 26 μ m) are scarcer than in the other extrinsic muscles. II₂₂ fibers predominate at around 30% (30 μ m) while the remaining groups accounted for 27% (II₁ 43 μ m) and 24% (II₁ 42 μ m). Distributions are scattered.

Pars longitudinalis superior m. hyoglossus: In longitudinal sections of the tongue from up to including the entire fiber courses of the extrinsic muscles (i.e. those parts outside the tongue mass) it was shown that the thin muscle sheet of long fibers running under the dorsal tongue epiglottis consists entirely of type II fibers. The fibers could be traced to those parts of the tongue root where fibers of m. hyoglossus pass, but no clusters of II fibers could be found there nor were there

Table 2 *Frequency and size of type I and type II fibers in the tongue muscles*

Muscle	Number of fibers	Frequency (%) of fiber types				Mean fiber diameter (μ m) and standard deviation (Sheppard's correction used)			
		I ₁₂	II	II ₁	II ₂₂	I ₂₂	II	II ₁	II ₂₂
<i>Extrinsic</i>									
Genioglossus	1 478	25.4	23.9	25.1	25.6	26.9 \pm 5.1	39.9 \pm 7.2	39.4 \pm 6.9	38.3 \pm 6.3
Hyoglossus	1 713	19.1	26.9	24.1	29.9	26.3 \pm 5.0	40.9 \pm 7.3	41.8 \pm 7.4	39.3 \pm 6.3
Styloglossus	1 214	22.8	27.0	22.3	27.9	29.7 \pm 6.4	40.9 \pm 7.3	38.7 \pm 7.1	31.4 \pm 6.4
<i>Intrinsic</i>									
Transversus	506	0.2	36.7	32.4	30.7	—	30.3 \pm 4.3	26.4 \pm 3.9	19.9 \pm 3.9
Verticalis	531	0.4	10.2	6.4	83.0	—	27.3 \pm 6.2	23.8 \pm 5.4	28.2 \pm 6.2
Pars long. sup. m. hyoglossus	483	—	16.8	66.9	16.3				
Pars long. inf. m. styloglossus	556	—	14.7	45.8	39.5				

as supported by grants from Karolinska Institute I wish to express my gratitude to Professor Carlsson for kindly allowing the use of the computer. The skilful technical assistance of Ms Hoppe is gratefully acknowledged.

REFERENCES

- VALEK, S. 1938. A contribution to the study of movements of the tongue in animals, with special reference to the cat. *J Anat (Lond)* 73, 15-30.
- H. T. J. 1968. Statistical methods in biology. *W. B. Saunders Co.*
- Y. M. 1967. ATPase activity of myosin correlated with speed of muscle shortening. *J Gen Physiol* 51, 1-18.
- R. D. J. W. 1940. The hypoglossal complex of vertebrates. *J Comp Neurol* 72, 489-574.
- R. D. J. J. EDGERTON, V. R. FURUKAWA, PETER, J. B. 1971. Histochemical biochemical contractile properties of red, white and intermediate fibers. *Am J Physiol* 220, 410-414.
- H. 1974. Anatomie des Menschen. II Band. *Springer Verlag*.
- J. E. M. B. & ENGEL, W. K. 1969. The histochemical analysis of human muscle biopsies with regard to fiber types. *Neurology* 19, 221-233.
- R. E. LEVINE, D. M. ZAJAC, F. M. 1971. Mammalian muscle biopsies: Physiological-histochemical correlation in types in cat gastrocnemius. *Science* 174, 709-711.
- ETON, H. M. & DRURY, M. A. S. 1957. Histochemical techniques. Oxford University Press, London.
- J. E. M. B. & BUTCHER, M. C. 1971. Practical Histochemistry. Wiley, London.
- E. R. I. 1972. Dynamic properties of mammalian skeletal muscles. *Physiol Rev* 52, 125-197.
- G. T. 1945. The effect of stimulation and recovery on the phosphorylase content of muscle. *J Biol Chem* 163, 331-339.
- QUINHAM, D. J. 1964. Textbook of zoology (ed. J. R. Krombein), 10th ed. London.
- LOW, R. 1951. Vorarbeiten zu einer Betrachtung der Zunge als funktionelles System. *Zeitschrift für Morphologie und Jährbuch* 91, 33-76.
- AN, G. A. 1973. Review of the evolution and phylogeny of the mammalian tongue. *Acta Anat* 91, 125-129.
- AN, G. A. & BAGGETT, H. 1971. A structural and functional classification of mammalian tongues. *J Anat* 107, 427-429.
- AN, G. A. & BAGGETT, H. 1972. The geniohyoid muscle: re-examination of its anatomy in some mammals, including man. *Acta Anat* 83, 403-410.
- NER, R. SESSLE, B. J. & STOREY, A. T. 1978. The neural bases of oral and facial function. *Plenum Press*, New York.
- KOFTITZ, V. & BROOKE, M. H. 1973. Muscle biopsies: A modern approach. W. B. Saunders Co., London.
- DUVE, C. de, WATTIAUX, R. & BAUDHUIN, P. 1962. Distribution of enzymes between subcellular fractions of animal tissues. *Advanc Enzymol* 4, 291-358.
- EDGEWORTH, F. H. 1935. The cranial muscles of vertebrates. Cambridge University Press.
- EDSTRÖM, L. & KUGELBERG, E. 1968. Histochemical composition, distribution of fibres and fatigability of single motor units. Anterior tibial muscle of the rat. *J Neurol Neurosurg Psychiat* 31, 424-433.
- EDSTRÖM, L. & LINDQUIST, Chr. 1973. Histochemical fiber composition of some facial muscles in the cat in relation to their contraction properties. *Acta Physiol Scand* 89, 491-503.
- EDSTRÖM, L., LINDQUIST, Chr. & MÄRTENSSON, A. 1974. Correlation between functional and histochemical properties of the intrinsic laryngeal muscles in the cat. In: Ventilatory and phonatory control systems (ed. M. Wyke) pp. 392-407. Oxford University Press, Oxford.
- FENICHEL, G. M. & ENGEL, W. K. 1963. Histochemistry of muscle in sphincter spina muscular atrophy. *Neurology* 13, 1059-1066.
- GRANT, R. & BURKE, R. E. 1973. The control of movement and posture. *Brain Res* 53, 1-28.
- GRAY, H. 1973. Anatomy (ed. R. Warwick & F. L. Williams) pp. 1237-1243. Longman Group Ltd, Edinburgh.
- HAYASHIMOTO, I. 1960. Comparative anatomical studies on the hypoglossal nucleus of the carnivore. *Bull Kobe Med College* 20, 394-408.
- HELLSTRAND, E. 1979. Histochemical morphological and functional properties of tongue muscles in cat. Abstracts from XVI Scandinavian Congress of Physiology and Pharmacology Oslo 1979. *Acta Physiol Scand Suppl.* 473, 37.
- HENNEMAN, E. & OLSON, C. B. 1965. Relations between structure and function in the design of skeletal muscles. *J Neurophysiol* 28, 581-598.
- HESSE, F. 1875. Über die Muskeln der menschlichen Zunge. *J Anat Entwicks-Gesch* 1, 80-106.
- HOLT, S. J. & WITHERS, R. F. 1958. Studies in enzyme cytochemistry. V. An appraisal of endogenous reactions for enzyme localization. *Proc Roy Soc B* 148, 520-532.
- KOSAKA, K. & JAQUITA, K. 1903. Experimentelle Untersuchungen über die Ursprünge des Nervus Hypoglossus und seines absteigenden Astes. *Arch Psychiat Neurol* 24, 190-199.
- KUGELBERG, E. 1973. Properties of the rat bioelectric motor units. In: New developments in electromyography and clinical neurophysiology (ed. J. E. Desmedt), vol. 1, pp. 2-13. Karger, Basel.
- KUGELBERG, E. & EDSTRÖM, L. 1968. Differential histochemical effects of muscle contractions on phosphorylase and glycogen in various types of fibres, relation to fatigue. *J Neurol Neurosurg Psychiat* 31, 415-423.
- LOWE, A. A. 1976. Neural mechanisms involved in tongue motility. Thesis, Univ. of Toronto.
- LUDWIG FERDINAND. 1884. Zur Anatomie der Zunge. *Lehr-artist. Anstalt Theod. Riedel, München*.
- MORIMOTO, T., KATO, I. & KAWAMURA, Y. 1966.

tem. The superior longitudinal muscle layer is defined as a fiber portion of *m. hyoglossus*. It consists wholly of type II fibers running in the dorsal layer, most of them originating in the same place as *m. hyoglossus*. A similar close connection between the hyoglossal and the longitudinal fibers at the tongue root was earlier reported by Dabelow 1951 (see also Nishi 1938). The peripheral connection found between the superior longitudinal fibers and the fibers of *m. hyoglossus* accords well with reports on the tongue motor nucleus in the central nervous system. In their study on dog and rabbit, Kosaka & Jagata (1903) pointed out that resection of the dorsal tongue half leads to retrograde degeneration of the dorsolateral part of the hypoglossal nucleus, an area they and subsequent investigators (Barnard 1940, Morimoto, Kato & Kawamura 1966) have shown to comprise the neurons innervating *m. hyoglossus*. Further, the comparative anatomical study of Hayashimoto (1960) suggested identity between the central projection of the retractive muscles and the innervation area of the superior longitudinal muscle. Relatedly, Barnard (1940) has suggested that the superior longitudinal muscle splits off from *m. hyoglossus* in embryo.

The inferior longitudinal muscle layer is here defined as a fiber portion of *m. styloglossus* composed solely of type II fibers and running in the ventral layer of the tongue. Its fiber course differs entirely from various earlier descriptions of *m. longitudinalis inferior* (e.g., Braus 1974, Abd-El Malek 1938, Cunningham 1964, Gray 1971). Previously the inferior muscle was said to extend from the ventral part of the tongue tip to the hyoid bone, in running between *m. genioglossus* and *m. hyoglossus* along the middle part of the tongue. The present study reveals that this middle region is composed of a mixture of fibers of types I and II from *m. genioglossus* and *m. hyoglossus*, both of which are laterally limited by the type I and II fibers of *m. styloglossus*. The lateralmost styloglossal fibers are all type II, however, forming a fiber portion that can be traced up to the ventral part of the tongue tip and back to the insertion points of *m. styloglossus*. The ventrolateral fiber course is quite in agreement with the anatomy of the lateral part of the styloglossal muscle described by Dabelow 1951. *M. styloglossus* receives its central projection from the dorsomedial part of the hypoglossal nucleus (Barnard 1940). The study by Morimoto, Kato & Kawamura (1966) suggests that that electrical

stimulation of this area leads to retractive lateral deviation of the ipsilateral tongue half, i.e., the tip which becomes ventroflexed.

The assumption that the longitudinal muscle portions are part of the retractive system has further support in the mean fiber sizes. The longitudinal fibers are 4 μ m in diameter to the type II fibers of the curvicles.

Studies of the histochemical properties of twitch muscles have revealed the presence of groups of muscle fibers, one of type I and type II, where all three occur the type I usually predominates (e.g., Stein & Padykula, Henneman & Olson 1965, Kugelberg & Burke 1968, cf. also reviews by Close 1970, Burke 1973). Recent studies on mammalian respiratory muscles confirm this fiber pattern (Taylor, Cody & Bosley 1973, Edström & Taylor 1973, Edström, Lindquist & Mårtensson 1975, also Dubner, Sessle & Storey 1978). The histochemical properties of the tongue muscles fit into this system. For instance, the fibers of I₁₂₃, II₁ and II₁₂₃ seem comparable to the I & II fibers of Stein & Padykula (1962). The fiber I₁₂₃ identified in this study constitutes the most of the hyoglossal and styloglossal fibers that enter into the tongue tip.

Type II fibers have a dense uniform load of mitochondria rich in oxidative enzymes, as a dense peripheral distribution of myofibrils, features that are essential structural correlates to resistance to fatigue (Edström & Kugelberg, Burke et al. 1971). The large amount of highly oxidative fibers in the tongue muscle confers considerable fatigue resistance. This characteristic has in fact been demonstrated in the skeletal muscles, which lose only about 10–25% initial force of their twitch tension during 10 min activity (Hellstrand 1979).

According to Bárány 1967 and Barnard et al. (see also Burke et al. 1971, Kugelberg 1977) related to myofibrillar ATPase activity, I contraction time. Since type II fibers are low in ATPase activity and type I fibers low in the muscles might be expected to contract more slowly than the intrinsic, which have almost all type I fibers. Indeed, the mean values of isometric contraction time reported for the two muscle groups are about 100 ms apart, the intrinsic muscles being the faster (Hellstrand 1979).

pancreatic polypeptide (APP) inhibits atropine ant vasodilation in cat submandibular ry gland and nasal mucosa ble interaction with VIP

LUNDBERG ANDERS ÄNGGÅRD TOMAS HÖKFELT and JOE KIMMEL

ns of Pharmacology and Histology Karolinska Institute, Stockholm, Sweden and McEwen Laboratory
rsity of Kansas Medical Center Kansas, USA

pancreatic nerve stimulation produces a
increase in blood flow from the subman-
alvary gland (Bernard 1858) and nasal mu-
: Änggård 1974) which unlike the secretory
s, is largely atropine resistant (Heldén &
Änggård 1974). The submandibular vaso-
has been attributed to activation of both
or nerves with a special cholinceptive
en (Dale & Gaddum 1930) and metabolic
on of a local system (Hüboe & Lewis 1956).
e of kinetic has, however, been questioned
et al. 1965). Recent studies indicate the
e of a potent vasodilatory agent, vasoactive
al polypeptide (VIP), in nerves of both the
dibular salivary gland (Bryant et al. 1976)
nasal mucosa (Uddén et al. 1978). The
rves are of parasympathetic origin (Änggård
1979, Lundberg et al. 1980a). Further mor-
ical analysis suggests that VIP is present in
tic neurones (Lundberg et al. 1979, 1980a).
le of VIP as a mediator of vasodilation in the
dibular salivary gland is further support-
its atropine resistant vasodilatory action
zu & Taira 1979, Bloom & Edwards 1980,
erg et al. 1980a). Furthermore, a marked
e of VIP in the venous effluent from the
dibular salivary gland is further support-
ic nerve stimulation (Bloom & Edwards 1980
erg et al. 1980). The vasodilation caused by
reshold nerve stimulation can partially be
el by infusion of VIP antiserum (Lundberg et
1980).

urther attempts to block the atropine resistant
ation, we have focused our interest on avian
c pancreatic polypeptide (APP). APP is a 36 amino
polypeptide (Kimmel et al. 1975), which great-

ly differs in amino acid sequence from pancreatic
polypeptide isolated from other species (Lin &
Chance 1974, Kimmel et al. 1975). Recently Lorén
et al. (1979) have described the distribution of
nerves displaying immunoreactivity to APP in brain
and gut. In addition this peptide or a structurally
related peptide is present in adrenergic nerves
around blood vessels in both the nasal mucosa and
submandibular gland of the cat (Lundberg et al.
1980b).

Experiments were done on the cat submandibular
salivary gland. The systemic blood pressure, local
blood flow (via drop counter) and salivation (via
drop counter) were recorded on a Grass polygraph.
Parasympathetic preganglionic nerve stimulation (6
Hz, 8 V, 1 ms) was performed on the chorda-lingual
nerve and sympathetic stimulation on the cervical
sympathetic trunk (7 Hz, 8 V, 1 ms). Close intra-
arterial infusions of APP dissolved in phosphate
buffered saline containing 0.5% bovine serum albu-
min was performed at a rate of 50 µl/min. For de-
tails see Lundberg et al. (1980a). The nasal blood
flow was studied in separate cats. Preganglionic
parasympathetic nerve stimulation (parameters as
above) was performed on the Vidian nerve in previ-
ously sympathectomized cats (Änggård 1974). The
cervical sympathetic trunk was stimulated in normal
animals (parameters as above). Close intraarterial
infusions were done at a rate of 100 µl/min. Atro-
pine (0.5 mg/kg) was given i.v.

Parasympathetic nerve stimulation caused a more
than 5-fold increase in submandibular blood flow
and a marked salivary secretion (Fig. 1a). Local
infusion of APP (0.1 or 1 µg/min) 2 min prior to and
during nerve stimulation caused a marked reduction
(70±8% \sim 4) of the vasodilatory response (Fig. 1

- Studies on functional organization of the hypoglossal nucleus. *J Osaka Univ Dent School* 6: 75-87.
- MOWRY R W & MILLICAN R C 1957 A histochemical study of the distribution and fate of dextran in tissues of the mouse. *Amer J Pathol* 28: 522.
- NACHLAS M M, TSOU K C, SOUZA de E, CHENG C S & SELIGMAN A M 1957 Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. *J Histochem Cytochem* 5: 420-436.
- NISHI H 1938 Muskeln des Kopfes. Panetale Muskulatur. In: *Handbuch der vergleichenden Anatomie der Wirbeltiere* Bd V (ed. Bolk et al.) Urban, Berlin, Wien.
- NUSBAUM J & MARKOWSKI Z 1896. Zur vergleichenden Anatomie der Stützorgane in der Zunge der Säugetiere. *Anat Anz* 1: 551-561.
- NYSTRÖM B 1968 Histochemistry of developing cat muscles. *Acta Neurol Scand* 44: 405-419.
- PADYKULA H A & HERMAN, E 1957 Specificity of the histochemical method for triphosphatase. *J Histochem Cytochem* 5: 71.
- PEARSE A G E 1960 *Histochemistry applied*. 2nd ed. J. & A. Churchill Ltd, London.
- REIGHARD J & JENNINGS H S 1961 *Is the cat*. Holt, Rinehart & Winston, New York.
- ROMEIS B 1968 *Microscopische Technik*. W. Oldenbourg Verlag, München.
- SCARPELLI D G, HESS R & PEARSE A G E 1958 The cytochemical localization of enzymes. I. *J Biophys Biochem Cytol* 4: 75-77.
- STEIN J M & PADYKULA H A 1957 Histochemical classification of individual skeletal fibers of the rat. *Am J Anat* 110: 105-124.
- TAKEUCHI T & KURIAKI H 1955 Reaction detection of phosphorylase in animal tissues. *J chem Cytochem* 3: 153-160.
- TAYLOR A, CODY F W J & BOSLEY H A 1954 Histochemical and mechanical properties of cat muscles of the cat. *Exp Neurol* 38: 99-109.

— gives a response very similar to the one fully described after infusion of VIP (Lundberg et al. 1980). It is therefore suggested that APP is a structurally similar peptide with mechanisms in exocrine glands related to VIP. Since a close morphological association between VIP and APP-immunoreactive material around blood vessels of the nasal mucosa and submandibular gland (Lundberg et al. in press), the inhibitory effect of APP on the vasodilatory response may be of physiological importance. The exact (postsynaptic) mechanism of inhibition, however, has to be established.

— This study was supported by grants from the Medical Research Council (17X/5434/04X/23877) Tobena Akterbolaget, Magnus Bergvalls Stiftelse and Alice Wäsenbergs Stiftelse. For skilful assistance we thank Mrs W. Hori.

REFERENCES

- ÅRD, A. 1974 The effect of parasympathetic stimulation on the microcirculation and secretion in the nasal mucosa of the cat. *Acta Otolaryng* 78: 105.
- ÅRD, A., LUNDBERG, J. M., HÖKFELT, T., JONSSON, G., FAHRENKRUG, J. & SAID, S. J. 1979 Inhibition of the cat nasal mucosa with special reference to relations between peptidergic and cholinergic neurons. *Acta Physiol Scand Suppl* 473: 30.
- ÅRD, C. 1858 De l'influence de deux ordres de fibres qui déterminent les variations de couleur du sang, dans deux des organes glandulaires. *CR hebdo Séanc Acad Sci Paris* 47: 245–257.
- BLA, K. D., MORLEY, J., SCHACHTER, M. & DALE, L. H. 1965 Vasodilatation in the submandibular gland of the cat. *J Physiol* 179: 172–184.
- CL, S. R. & EDWARDS, A. V. 1980. Vasodilatory peptide in relation to atropine resistant vasodilation in the submandibular gland of the cat. *J Endocr* 308: 41–53.
- ANT, M. G., BLOOM, S. R., POLAK, J. M., ALQUERQUE, R. H., MODLIN, J. & PEARSE, A. E. 1976 Possible dual role for vasoactive intestinal peptide as gastrointestinal hormone and neurotransmitter substance. *Lancet* 991: 993.
- DALE, L. H. & GADDUM, J. H. 1930. Reactions of denervated olfactory muscle and their bearing on the mode of action of parasympathetic and related nerves. *J Physiol* 70: 109–144.
- HEIDENHAIN, R. 1872. Ueber die Wirkung einiger Gifte aus dem Nerven der glandula submandibularis. *Pflügers Arch* 3: 309–318.
- HILTON, S. M. & LEWIS, G. P. 1956 The relationship between glandular activity, bradykinin formation and functional vasodilatation in the submandibular salivary gland. *J Physiol* 134: 471–483.
- KIMMEL, J. R., HAYDEN, L. J. & POLLOCK, H. G. 1975 Isolation and characterization of a new pancreatic polypeptide hormone. *J Biol Chem* 250: 9369–9376.
- LIN, T. M. & CHANCE, R. E. 1974 Gastrointestinal actions of new bovine pancreatic polypeptide (BPP). In: *Endocrinology of the gut* (ed. W. Y. Chey & F. P. Brooks), pp. 143–145. C. R. Slack Inc., New Jersey.
- LORÉN, I., ALUMETS, J., HÅKANSON, N. R. & SUNDLER, F. 1979 Immunoreactive pancreatic polypeptide (PP) occurs in the central and peripheral nervous system. Preliminary immunocytochemical observations. *Histochemistry* 78: 179–186.
- LUNDBERG, J. M., ÅNGGÅRD, A., FAHRENKRUG, J., HÖKFELT, T. & MUTT, V. 1980 Vasoactive intestinal polypeptide (VIP) in cholinergic neurones of exocrine glands: Possible functional significance of co-existing transmitters for vasodilatation and secretion. *Proc Natl Acad Sci US* 77: 1651–1656.
- LUNDBERG, J. M., HÖKFELT, T., ÅNGGÅRD, A., KIMMEL, J., GOLDSTEIN, M. & MARKEY, K. 1980 Coexistence of an avian pancreatic polypeptide (APP) immunoreactive substance and catecholamines in some peripheral and central neurones. *Acta Physiol Scand* 110: 107–109.
- LUNDBERG, J. M., HÖKFELT, T., SCHULTZBERG, M., UYNÄS-WALLENSTEIN, K., KÖHLER, C. & SAID, S. 1979 Occurrence of vasoactive intestinal polypeptide (VIP)-like immunoreactivity in certain cholinergic neurones of the cat: Evidence from combined immunohistochemistry and acetylcholinesterase staining. *Neuroscience* 4: 1539–1559.
- SHIMIZU, T. & TAIRA, N. 1979 Assessment of the effect of vasoactive intestinal peptide (VIP) on blood flow through and salivation of the dog salivary gland in comparison with those of secretion of glucagon and acetylcholine. *Br J Pharmacol* 65: 683–688.
- UDDMAN, R., ALUMETS, J., DENBERT, O., HÅKANSON, N. R. & SUNDLER, F. 1978 Occurrence and distribution of VIP nerves in the nasal mucosa and tracheobronchial wall. *Acta Otolaryngol* 86: 443–448.



Fig 1 *a b* Effects of parasympathetic nerve stimulation (6 Hz, 2 min) on cat submandibular gland blood flow and salivary secretion before (*a*) and after (*b*) local infusion of APP (0.1 μ g/min). BF = blood flow (every 10th drop is separately indicated). BP = systemic blood pressure (140 mmHg). S = salivation (every drop is separately indicated). T = time in min.

b) Secretion was also reduced to a smaller extent ($50 \pm 6\%$, $n=4$) (Fig 1 *a b*). 30 min after APP infusion no certain inhibitory effect could be recorded. The sympathetic response was not affected by the APP infusion (Fig 2 *a* and *b*).

In the nasal mucosa parasympathetic nerve stimulation caused an almost 3-fold increase of local blood flow, which was largely atropine resistant. Local infusions of APP (0.1 μ g/min) reduced this vasodilatory response ($80 \pm 7\%$, $n=4$). The blockage was reversible. The effect of sympathetic nerve stimulation was not influenced by APP.

In agreement with previous studies on the submandibular gland VIP (0.1 μ g/min) caused a marked vasodilation (Fig 3 *a*). This effect was markedly re-

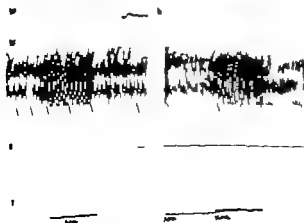


Fig 2 *a b* Effects of sympathetic stimulation (6 Hz, 2 min) on the parameters described in Fig 1.



Fig 3 *a b* Effects of local VIP infusion (0.1 μ g/min) prior to and after APP pretreatment (1 μ g/min) for 1 min.

duced ($90 \pm 10\%$, $n=4$) by infusion of APP (0.1 min) prior to and/or during the VIP administration (Fig 3 *b*). On the other hand, the effect of acetylcholine (0.1 μ g/min) was not reduced by APP (0.1 and 1.0 μ g/min) (Fig 4 *a* and *b*).

APP in concentrations up to 100 μ g/min did not influence blood flow in salivary gland mucosa, nor could any effects on systemic pressure be recorded.

The present findings suggest that APP selectively inhibits the nervously induced atropine-resistant vasodilation both in the submandibular gland and the nasal mucosa. Also the secretory inhibition, possibly via an effect on blood flow, never interaction with cholinergic mechanisms, not be excluded. APP seems to selectively inhibit the parasympathetic response, since the sympathetic vasoconstriction was not reduced.

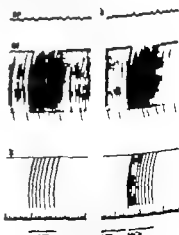


Fig 4 *a b* Effect of local acetylcholine infusion (0.1 μ g/min) prior to and after APP treatment (1 μ g/min) for 1 min.

nous constriction during cervical sympathetic ation in the cat

M AUER and BARBRO B JOHANSSON

Clinic of Neurosurgery Graz, Austria and Department of Neurology
of Göteborg, Sweden

tical importance of the adrenergic in-
of cerebral arterial vessels has been the
f considerable debate (see Edvinsson &
de 1976). By contrast, the nervous in-
in cerebral veins has attracted little atten-
of the fact that adrenergic nerve termi-
nals have been demonstrated in intracranial veins
(et al 1974). Whereas several reports deal
with arterial diameter changes during sympathetic
stimulation, there is no corresponding study concern-
ing cerebral veins. In the present study the re-
sponse of venous vessels to preganglionic cervi-
cal sympathetic stimulation was therefore studied
using intravital microscopy and a multi-
channel videoangiometer (Auer 1978, Auer &
1979).

Experiments were performed in 8 cats with a
weight of 1.5-3 kg. The animals were anesthe-
tized with 30 mg/kg sodium pentobarbital im-
jected with 6 µg/kg picrotoxin-bromide
endotracheally and respiration was controlled
with a 3 l/min flow of N_2O/O_2 . Body temperature was con-
stantly controlled with Philips rectal thermos-
tats and maintained at 37°C-38°C by aid of a
heating pad. Mean arterial pressure was continuous-
ly monitored via a PVC catheter inserted into the
descending aorta through the left femoral artery.
The internal jugular vein was cannulated for the eventual
infusion of bicarbonate solution in order
to maintain acid-base balance. The right femoral ar-
tery was cannulated for frequent blood gas analysis
(pH check). The right cervical sympathetic
nerve was isolated and sectioned below the superior
cervical ganglion and the cranial end mounted
on silver electrodes fixed into a plastic tube.
The vessel were then brought into a prone posi-
tion and the head was fixed in a stereotaxic head-
rest. A parietal cranial window with diameter
10 mm lateral to the sympathetic preparation

was made closed with a glass shield and sealed
with acrylic (Auer 1978).

Cerebral venous vessels were investigated through a
Leitz intravital microscope and the data were eval-
uated by aid of a multichannel video-angiometer
(Auer & Hayda 1979). Single experiments were
monitored with a TV-system and stored on video
tape. Measuring lines—freely movable on the TV
screen—could then be placed on any wanted vessel
portion to obtain continuous data concerning the
vessel diameter variations expressed in micrometer
(µm). Multiple replay of experiments on tape thus
allowed analysis of a large number of vessel seg-
ments. The sympathetic nerve was stimulated for
90 s with square wave pulses of 10 ms duration at
15 Hz so as to produce maximal pupil dilatation
and retraction of the membrana nictitans (1-10 V).

The resting mean arterial pressure was 118 ± 7
mmHg (SE). No significant change was noted dur-
ing sympathetic stimulation. $PaCO_2$ remained con-
stant at 29.6 ± 0.4 mmHg and PaO_2 at 101 ± 3 mmHg
throughout the experiments. Continuous record-
ings of venous caliber changes were performed
from 103 vessel segments with resting diameters be-
tween 32 and 486 µm (mean vessel calibre $141 \pm$
 9.4 µm). Pial veins started to constrict 9.1 ± 1.1 s af-
ter the onset of stimulation. Maximal constriction
was in most instances reached at 90 s or even some-
what later when stimulation had already been dis-
continued. However, in 11 vessel segments maxi-
mal constriction was observed after 40-80 s. In 5
vessel portions a slight dilatation was observed (see
Fig. 1). No consistent tendency could be demon-
strated when the time course of reactions of smaller
and larger veins was compared at the highest paper
speed of a multichannel penwriter. Statistical com-
parison between vessels with diameters up to 150
µm and those above 150 µm showed no significant
difference of the time reaction pattern: veins < 150

of veins during sympathetic stimulation comment to a figure by Forbes (1928) vein did not show measurable change. Constriction of larger veins obtained in the presence of about 20% would have an important effect on the blood volume. The physiological importance of the present findings remains to be seen. Spontaneous alterations of the sympathetic activity during physiological as well as pathological conditions. However the present study shows that possibility for changes in cerebral volume by alterations in sympathetic activity at least which is in agreement with histological studies (Owman *et al.* 1974).

This study was supported by the "Östermunchner Fonds för medicinsk forskning" (the Swedish Medical Council (projects No. 14X-4468)) and the Swedish Association against Heart and Chest Diseases.

REFERENCES

- AUER, L. M. 1978. The pathogenesis of hypertensive encephalopathy. *Acta Neurochir* Suppl. 27.
- AUER, L. M. & HAYDN, F. 1979. Multichannel videoangiography for continuous measurement of pial microvessels. *Acta Neurol Scand* 60, Suppl. 72: 208-209.
- EDVINSSON, L. & MACKENZIE, E. T. 1976. Amino acid mechanisms in the cerebral circulation. *Pharmacol Rev* 28: 275-343.
- EDVINSSON, L., LINDVALL, M., OWMAN, C. & WEST, K. A. 1980. Autonomic nervous control of cerebrospinal fluid production and intracranial pressure. In *Neurobiology of cerebrospinal fluid* (ed. J. H. Wood) Plenum Press, New York. In press.
- EDVINSSON, L., NIELSEN, K. C., OWMAN, C. & WEST, K. A. 1973. Evidence of vasoconstrictor sympathetic nerves in brain vessels of man. *Neurology* 23: 73-77.
- FORBES, H. S. & WOLFF, H. G. 1928. Cerebral circulation III. The motor control of cerebral vessels. *Arch Neurol Psychiat* 19: 1057-1086.
- MELLANDER, S. & JOHANSSON, B. 1968. Control of resistance, exchange and capacitance vessels in the peripheral circulation. *Pharmacol Rev* 20: 117-196.
- OWMAN, C., EDVINSSON, L. & NIELSEN, K. C. 1974. Autonomic neuroreceptor mechanisms in brain vessels. *Blood Vessels* 11: 2-31.

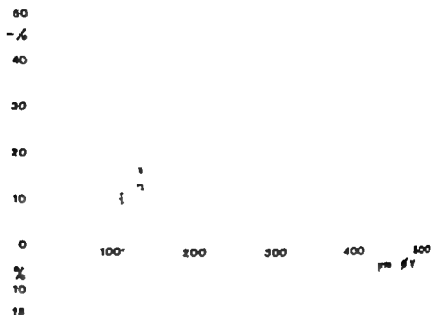


Fig. 1 Percentual pial vasoconstriction during sympathetic stimulation plotted against resting caliber. Single smaller branches show slight dilatation but basically there is a clear tendency to marked vasoconstriction with veins $>150 \mu\text{m}$ resting diameter reacting stronger than smaller ones.

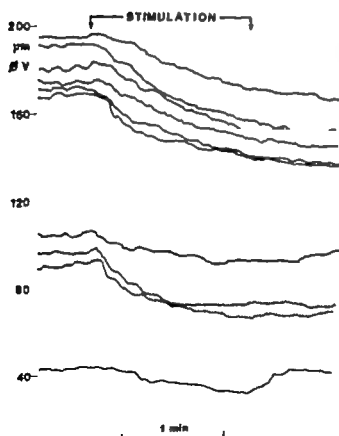


Fig. 2 Multichannel videoungdometric recordings of pial venous reaction during sympathetic stimulation. Venous diameter is given on the ordinate.

μm resting diameter started to constrict $10.8 \pm 1.6\%$ those $>150 \mu\text{m}$ within $7.2 \pm 0.6\%$.

Veins with a resting diameter $>150 \mu\text{m}$ reacted more than smaller veins. The percentual constriction is plotted against resting diameter. Mean constriction of veins $\leq 150 \mu\text{m}$ resting diameter was $11.5 \pm 0.9\%$ and of veins $>150 \mu\text{m}$ 1.9% (Student's *t* test $P < 0.0005$ for both) when compared to resting levels. Individual examples of diameter changes during sympathetic stimulation are shown in Fig. 2.

Considering the importance of blood volume in the intracranial pressure and the fact that the venous compartment contains about 80% of the total blood volume, it is surprising that so little attention has been given to the regulation of the veins. The tone of the veins is mainly regulated by sympathetic nervous activity in other vascular beds (Mellander & Johansson 1968). Sympathetic stimulation has been shown to reduce the total blood volume in mice (Edvinsson et al. 1977), a sympathetic influence on intracranial pressure is well documented, an effect that could be mediated via changes in blood volume and/or via alterations in the secretion of cerebrospinal fluid (Edvinsson et al. 1980).

The only previous study we have for

Ine sensitive contractile motor effects stance P on the feline pylorus tomach in vivo

J. J. M. LUNDBERG, P. LINDBERG, A. DAHLSTRÖM
AHLMAN

Department of Surgery III and Institute of Neurobiology, University of Göteborg
Department of Histology, Karolinska Institute, Stockholm, Sweden

In Motility stomach, pylorus, substance P and cat

sence of gut stimulating depressor sub-
s intestine and brain, later called Substance
was originally reported by Euler & Gaddum
The contraction of intestinal smooth muscle
is distinguished from the action of ACh

as not inhibited by atropine *in vitro*. The
factor was later demonstrated to be an 11
acid peptide (Chang et al. 1971) identical with
a, which caused an atropine-resistant in-
of salivation *in vivo* in rats (Lorenson &
crickling 1967).

is widely distributed throughout the body
early in the central nervous system and the
crow 1953). By the use of immunohisto-
try SP was localized not only to various
of the brain but also to primary sensory
is at the spinal ganglia, the dorsal root and
ral horns of the spinal grey matter (Hökfelt et
77). In the mammalian gut, the highest con-
centrations were found within the duodenal and
wall, where SP in some species was local-
s populations of serotonin-containing entero-
talia cells (Pearse & Polak 1973). By immuno-
chemistry SP was also demonstrated within
cell bodies and terminals of the intramural
s (Schultzberg et al. 1980).

has been regarded as sensitive neurotrans-
het in several biological test systems this has
questioned because of the slow onset and long
on of its effects (cf. Polak & Bloom 1979). The
ence of SP in dorsal roots and spinal cord has
the assumption of SP as a central excitatory
mitter of some primary afferent neurons, sup-
d by the electrophysiological findings of Ot-

saka et al. (1975). The functional importance of SP
in the peripheral nervous system has been focused
on antidromic vasodilatation (cf. Lembeck et al.
1979), but the localization of SP in nerve terminals
not only around blood vessels but also along
smooth muscle cells and in intramural ganglia of the
gut (Hökfelt et al. 1977) may indicate other func-
tions as well (Katayarova et al. 1979). Immunohis-
tochemical studies in the cat further suggest a
more dense SP innervation of the gastrointestinal
sphincters than of other gut regions (Lundberg et al.
1980).

The purpose of the present study was to record
the motor effects of local i.a. administration of SP
on the feline pylorus and stomach of anesthetized
cats *in vivo*.

11 cats in chloralose anesthesia (50 mg/kg b.w.)
were used. Intraarterial blood pressure and gastric
and pyloric motility were continuously recorded.
Gastric motility was studied by volume recordings
of a flaccid intragastric balloon at constant intra-
luminal pressure (Martinsson 1965). Pyloric motility
was recorded as changes of an applied constant
flow of body warm saline across the sphincter (Edlin
1980). A fine heparinized plastic catheter (21 mm)
was introduced towards the antro-pyloric region via
the splenic artery in retrograde direction. Ink in-
fusions after the experiments demonstrated a re-
gional distribution to the upper part of the duo-
denum, the pylorus and the gastric antrum-corpus.
SP (Peninsula, Ca. USA) was dissolved in saline
and given as a single bolus dose of 0.5–1 ml volume
(0.5–4 µg) via this catheter. SP injected i.a. induced
prompt powerful contraction of the stomach and

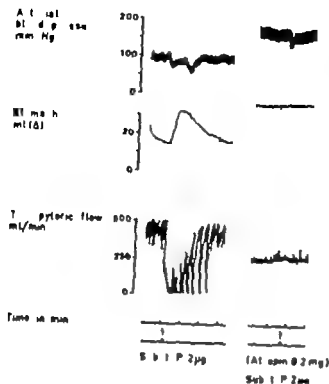


Fig. 1 The effects of regional i.a. injection of SP on intra-arterial blood pressure, gastric motility and transpyloric flow in the anesthetized cat before and after the administration of atropine i.v. After atropine the effect on blood pressure was not completely blocked while both motor effects were totally abolished.

the pylorus of 2–4 min duration in all animals. A slight reduction of the blood pressure of similar duration was also obtained (Fig. 1). The duration of these effects was dose-dependent. In the pylorus even the low doses caused a total but comparatively short cessation of the transpyloric flow initially. No gradation of the gastric contractile response was demonstrated. However only the four doses 0.5, 1, 2 and 4 µg of SP were tested in one and the same animal. 10 cats were given atropine (0.2 mg/kg i.v.) before SP was tested again. The effects on motility caused by SP were blocked in 9 of these 10 atropine pretreated cats (Fig. 1) while it induced a short lasting (<1 min) 50% decrease but no cessation of the transpyloric flow in one animal. However the SP induced decrease in blood pressure was not totally blocked by atropine (Fig. 1). It has earlier been demonstrated that this dose of atropine does not block the pyloric motor responses obtained after enkephalin and VIP i.a. (Edin 1980). The specificity of the atropine blockade was demonstrated by i.a. injections of ACh (100 µg). After atropine the ACh induced contractions were totally abolished.

Thus the present study demonstrates that SP administered via a regional artery elicits powerful contractions of the stomach and pylorus of a few minutes duration. Since SP immunoreactivity has been demonstrated both in ganglion cells of the two intramural plexuses surrounding nerve terminals of the pylorus and in the stomach, the possibility of SP as a neurotransmitter in the gut should be considered. Furthermore, axons in the vagal nerve contain like immunoreactivity demonstrated after the nerve at the abdominal level (Lundberg 1980). These fibres may originate at least partly from cell bodies of the nodose ganglion demonstrated to contain many SP-positive cells (Lundberg pers. comm.). Surprisingly, however, in these experiments the motor effects of SP were blocked by atropine in contrast to most *in vitro* studies. This suggests that the contractor effects of SP observed in this experimental *in vivo* model probably involves the activation of cholinergic neurons, presumably located in the intramural plexuses. In the living animal the effect by SP may either be accomplished via intramural SP neurons or via sensory SP fibres in the vagal or splanchnic nerves (cf. Lundberg 1980). Since the vagally induced pyloric contraction studied by this model is atropine resistant but sensitive to hexamethonium and naloxone (1980), vagal sensory SP fibres seem less likely to mediate the effect observed in this study.

This study was supported by grants from G. Läkareidiskap, the Medical Faculty of Göteborg and Swedish Medical Research Council (17X 523, 64 04P-4173).

REFERENCES

- CHANG M. M., LEEMAN S. E. & NIALL, R. P. 1982. Amino acid sequence of substance P. *Nature* **292**, 86–87.
- EDIN, R. 1980. The vagal control of the pyloric function. A physiological and immunohistochemical study in cat and man. *Acta Physiol. Scand. Suppl.*
- EULER, U. S. & GADDUM, J. H. 1971. A newly identified depressor substance in certain autonomic nerves. *J. Physiol. (Lond.)* **72**, 74–87.
- HÖKFELT, T., JOHANSSON, O., KELLER, L., LUNDGAHL, Å., NILSSON, G., NYGÅRD, P. & PERROW, B. 1977. Immunohistochemical demonstration of substance P in Substance P. Nobel-Symposium 37 (ed. U. S. von Euler & B. Pernow) 117–126. Press, New York.

Sensitivity of perfused fat cells to the antilipolytic effect of adenosine

L. LEVI

Department of Pharmacology, Karolinska Institute, Stockholm, Sweden

Adenosine may be an important modulator of lipolysis in adipose tissue (cf. Schwabe et al. 1975; Hjendahl et al. 1978). Adenosine accumulates during lipolysis in adipocytes and is a potent inhibitor of lipolysis in isolated fat cells (Faust et al. 1972; Ebert & Schwabe 1973; Stock & Prinsop 1976; Hjendahl & Fredholm 1976). The accumulation of adenosine in adipocytes is prevented by perfusion of fat cells (Allen 1973) since the cells are only exposed to fresh perfusate. The antilipolytic effect of adenosine on fat cells has been studied with this technique (Turpin et al. 1977; Hjendahl & Sollevi 1978). However, the antilipolytic potency of adenosine was more than 100-fold in the work by Turpin et al. (1977) than in our study. Turpin et al. (1977) reported that a 10 nM solution of adenosine reduced a submaximal lipolysis response to adrenaline by 50% whereas we found a 25% inhibition by 1 μ M adenosine. Basal plasma adenosine levels are close to 1 μ M (Fredholm & Sollevi 1980). The results of Turpin et al. (1977) imply an almost maximal effect already under basal conditions, while our results suggest that the effect of adenosine has to be raised above basal to elicit an antilipolytic effect. To understand the effect of adenosine in the regulation of lipolysis it is therefore important to clarify which of the estimates of antilipolytic potency is correct.

Isolated fat cells. Cells were obtained from decapitated male Sprague-Dawley rats (Arvidsson) weighing between 150-450 g (200 g). Isolated fat cells were prepared, using 3 mg/ml crude bacterial collagenase (Worthington Biochemicals) after the final washing. 1 ml aliquots of packed cells were transferred to temperature-controlled plastic tubes containing 2-2.6 ml Krebs-Ringer phosphate buffer, pH 7.4, with half the recommended calcium concentration, 5.5 mM glucose and 1% bovine serum albumin (cf. Loven). The floating fat cells were sub-

sequently "perfused" (Allen et al. 1973) with this buffer at 37°C and a rate of 2 ml/min. Drugs were infused via side-arm before the chamber. Lipolysis was measured as glycerol production rate (nmol/min/cell). Glycerol was measured by the method of Laurell & Tjallingii (1966), but the $ZnSO_4$ - $Ba(OH)_2$ precipitation step was eliminated as in the previous study (Hjendahl & Sollevi 1978) to increase the sensitivity. Standard curves for glycerol in the perfusion buffer gave expected values despite this modification. The glycerol assay was not influenced by the drugs administered.

The perfusates were collected in ice-cooled plastic tubes and immediately frozen thereafter. When thawed the samples (25-100 μ l) were immediately and without purification analyzed for adenosine and inosine by high performance liquid chromatography. A Waters model 6000A solvent delivery system and Waters U6K injector was used. The separation was performed on Waters μ -Bondapak C_{18} column protected with 40 μ m precolumn packed with the same material. The mobile phase (0.01 M NH_4PO_4 , pH 6.0 containing 14% methanol) was delivered at 1.5 ml/min. Absorbance was continuously monitored at 254 nm by Waters model 440 absorbance detector and recorded on Kipp and Zooner recorder. Peak heights in these chromatograms are linearly related to the amount of adenosine, inosine and other purines over a wide range (2-100 pmol) and were therefore used for quantification.

There was a basal outflow of glycerol from the isolated perfused fat cells of 8.3 ± 1 nmol/ml cells⁻¹ min⁻¹ (n=12). Adenosine and inosine concentrations in the perfusate were below the detection limit (5-10 nM) during both basal and stimulated conditions, indicating a very low adenosine level in the medium surrounding the cells. In keeping with the concentration of adenosine required to inhibit lipolysis and the adenosine levels found in the perfusate of our system, we previously have not (Hjendahl & Sollevi 1978) found that adenosine deaminase (ADA) enhances lipolysis under these conditions. During adenosine infusion (1 μ M) glycerol outflow was 7.3 ± 1 nmol/ml cells⁻¹ min⁻¹ (n=7) which was not significantly different from the basal lipolysis (see also Turpin et al. 1977).

the effect of adenosine would have to be mediated by mechanisms keeping the adenosine level lower than those commonly encountered in tissues and body fluids. Such a high level, for example not compatible with basal levels in the 10^{-6} M range, since that would make fat cells virtually unresponsive to stimulation which increases the levels of cAMP to the μ molar range. On the other hand, these results confirm our previous estimate that antilipolytic effects of adenosine require adenosine concentrations in the μ molar range. Under adrenergic adenosine levels are probably too low to significantly affect lipolysis and only when raised by drugs or by adrenergic stimulation (Fredholm & Sollevi 1977; Fredholm & Sollevi, in manuscript) are antilipolytic concentrations reached.

This work is supported by the Swedish Medical Research Council (proj. no. 04X 2353) and by Karolinska

REFERENCES

FAIN, J. N., LAIBINIS, E. E., MILLER, E. A. & LAMORE, J. 1973. Continuous monitoring of lipolysis in perfused isolated fat cells. *J. Appl. Physiol.* 35, 1-17.
 FAIN, J. N. & SCHWABE, U. 1973. Studies on the antilipolytic effect of adenosine and related compounds in isolated fat cells. *Neuro-Schmidberg's Arch. Pharmacol.* 278, 247-259.
 FAIN, J. N. & MALBON, C. C. 1979. Regulation of adenylate cyclase by adenosine. *Mol. Cell Biochem.* 25, 1-131.

FAIN, J. N., POINTER, R. H. & WARD, W. F. 1972. Effects of adenosine nucleosides on adenylate cyclase, phosphodiesterase, cyclic adenosine monophosphate accumulation and lipolysis in fat cells. *J. Biol. Chem.* 247, 6866-6872.
 FREDHOLM, B. B. 1978. Effect of adenosine, some analogues and related drugs of lipolysis in rat fat cells. *Acta Physiol. Scand.* 102, 191-196.
 FREDHOLM, B. B. & SOLLEVI, A. 1977. Antilipolytic effect of adenosine in dog adipose tissue *in situ*. *Acta Physiol. Scand.* 99, 254-256.
 FREDHOLM, B. B. & SOLLEVI, A. 1978. Control of lipolysis and circulation in adipose tissue by endogenous adenosine. *Acta Physiol. Scand.* 102, 77A-78A.
 FREDHOLM, B. B. & SOLLEVI, A. The release of adenosine and inosine from canine subcutaneous adipose tissue by nerve stimulation and noradrenaline. *In manuscript*.
 HJEMDAHL, P. & FREDHOLM, B. B. 1976. Cyclic AMP-dependent and independent inhibition of lipolysis by adenosine and decreased pH. *Acta Physiol. Scand.* 96, 170-179.
 HJEMDAHL, P. & SOLLEVI, A. 1978. Antilipolytic effect of adenosine in isolated perfused fat cells. *Acta Physiol. Scand.* 103, 270-274.
 LAURELL, S. & TIBBLING, G. 1966. An enzymatic fluorimetric micro-method for the determination of glycerol. *Clin. Chem. Acta* 13, 317-322.
 SCHWABE, U., EBERT, R. & ERBLER, C. 1975. Adenosine release from fat cells. Effect on cyclic AMP levels and hormone actions. *Adv. Cyclic Nucleic Res.* 5, 569-584.
 STOCK, K. & PRILOP, M. 1974. Demonstration of catecholamine-induced formation of adenosine 3', 5'-monophosphate and release of glycerol in fat cells by prostaglandin E_1 , E_4 and N^6 -phenylethyladenosine. *Neuro-Schmidberg's Arch. Pharmacol.* 282, 15-31.
 TURPIN, B. P., DUCKWORTH, W. C. & SOLOMON, S. S. 1977. Perfusion of isolated rat adipose cell. Modulation of lipolysis by adenosine. *J. Clin. Invest.* 60, 442-448.

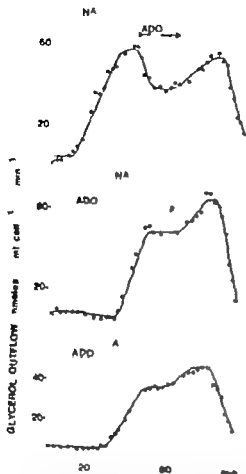


Fig. 1 Three typical experiments showing the antilipolytic effect of $1 \mu\text{M}$ adenosine (ADO) in perfused rat fat cells. In the upper figure adenosine was introduced during stimulation with noradrenaline (NA $0.13 \mu\text{M}$) and caused 31 ± 7 ($n=5$) per cent inhibition of glycerol outflow. In the middle figure adenosine was introduced prior to stimulation thereby inhibiting glycerol outflow by 2.5 ± 5 ($n=4$) per cent. When adrenaline (A $0.3 \mu\text{M}$) was the stimulating agent (lower figure) adenosine inhibited glycerol outflow similarly by 4 ± 3 ($n=3$) per cent.

A submaximally effective concentration of adrenaline ($0.3 \mu\text{M}$) which was used as lipolytic stimulus by Turpin et al. (1977) was equally potent as noradrenaline ($0.3 \mu\text{M}$) in stimulating lipolysis in our system, the glycerol production rate being 33 ± 5 ($n=3$) vs. 36 ± 4 ($n=9$) nmol/ml cells/min respectively. Noradrenaline and adrenaline stimulated lipolysis was equally affected by the addition of adenosine and the inhibition produced was of the same order of magnitude as that reported previously by us (Fig. 1) (Hjelm Dahl & Sollevi 1978). Thus the difference in choice of lipolytic agent can not explain the marked difference in antilipolytic potency of adenosine found in the two conflicting studies on perfused fat cells (Turpin et al. 1977



Fig. 2 A chromatogram showing the presence of adenosine and inosine in the effluent perfusate during adenosine infusion—solid line. The dashed line represents a standard solution containing adenosine and inosine respectively which was used as the sample. The concentration in the sample was $0.8 \mu\text{M}$ adenosine and $0.2 \mu\text{M}$ inosine.

Hjelm Dahl & Sollevi 1978). Another difference between these studies was the order in which adenosine and lipolytic agent were introduced. In our hands adenosine was equally potent in inhibiting lipolysis whether introduced before or after stimulation (Fig. 1).

It is known that bovine albumin is coated with various amounts of adenosine deaminase (ADA) capable of degrading exogenously added adenosine. Fain & Malbon (1979) found that the less pronounced antilipolytic effect of adenosine found by us (Hjelm Dahl & Sollevi 1978) could be due to extensive degradation of adenosine by ADA activity in the albumin coating of the fat cells in the same range as that reported by Turpin et al. (1977). However, the adenosine and inosine concentrations in the effluent perfusate during adenosine infusion gave values of 0.8 ± 0.1 and $0.7 \pm 0.1 \mu\text{M}$ respectively, indicating degradation of less than 25% of the adenosine under the present conditions. A typical chromatogram from one of the experiments is shown in Fig. 2.

Thus the present results do not offer an explanation for the very high antilipolytic potency of adenosine reported by Turpin et al. (1977). For the strain of rats used by these authors it is more sensitive. However, high sensitivity

Structurally reduced compliance of the venous capacitance vessels in spontaneously hypertensive rats (SHR)

SON and B. FOLKOW

of Physiology, University of Göteborg, Sweden

ence of cardiac, arterial and arteriolar wall play in primary hypertension was recognized a century ago, though the important consequences were realized only recently (Folkow 1978). Far less is known about pressure capacitance sections, where structural adaptation to pressure alternations occur almost with different hemodynamic forces. Thus, while media hypertrophy for its reasons amplifies precapillary resistances, such consequences are negligible in the thin-walled capacitance vessels. Instead, thickening should here mainly increase constrictor and/or reduce wall compliance, with important consequences for cardiac and overall hemodynamics.

Force of local load and muscle activity—the stimuli for wall hypertrophy—often seem to also in the low-pressure cardiovascular section of primary hypertension. For example, the altered autonomic innervation in neurogenic hypertension includes vasoconstriction causing blood centralization and pressure elevation in low-pressure parts (Julius & Esler 1975). Here left ventricular hypertrophy develops both for an increased filling pressure (cf. Folkow 1978). Furthermore, even distal venous pressures seem to be elevated to judge from signs of distal capillary pressure elevation (Ulrych 1979; Lundin & Folkow 1978).

Increasing mean both volume (Ulrych 1979) and venographic studies (Takeshita & Mark 1979) show a reduced venous distensibility in primary venous pressure, present also after constrictor fibre ablation. Though a structural compliance reduction likely to contribute, it is in man difficult to include non-neurogenic increases of venous muscle tone. In rat primary hypertension, complete vascular relaxation is easy to obtain. Simon's findings (1976) suggest structurally

reduced venous compliance. Here, however, the initial venous volume was not known, which is necessary for estimations of wall compliance, and the retrograde filling technique used might have unpredictably delimited the venous segments distended because of distal valves.

In this laboratory the relationships between altered cardiovascular design and hemodynamics in normotension and hypertension has been much studied (Folkow 1978). Particularly the rat hindquarter vascular bed has here been used for estimations of pre- and postcapillary resistances, capillary permeability and capillary volume (Kamada, Rippe & Folkow 1979). It is therefore a suitable target for precise estimations of venous volume and wall distensibility as well.

The experimental approach was based on the isogravimetric technique described elsewhere (for ref. see Folkow 1978). Briefly, isolated hindquarters from 10 pairs of matched spontaneously hypertensive rats (SHR) and Wistar normotensive control rats (NCR) (6-7 months of age, differing about 50% in mean arterial pressure) were perfused with constant flow at papaverine-induced maximal vasodilatation during continuous measurements of tissue weight and of inflow and outflow pressures (P_i and P_o). They were subsequently used also for other purposes. In the initial control situation P_i and P_o were set to isogravimetry but with P_i at 4 mm Hg to avoid both venous semicollapse and true wall distension. A 'the isogravimetric capillary pressure (P_{ca}) can be measured as well, the control pressures at both ends of the venous capacitance section (P_i and P_o) could be defined. In identical hindquarter preparations total intravascular volume present in this control situation, was deduced from flow and mean transit time (cardiographic) data.

From this control level known P_i elevations (and hence known P_o elevations) were induced during constant flow with measurements of the increased intravascular contents as derived from the initial rapid slope of weight increase. Thus, the relationships between average venous pressure increment

$$\Delta P = \Delta \left(\frac{P_i + P_o}{2} \right)$$

protein escape in SHR (Rippe, Lundin & Kamaya 1978) and hypertensive man (Ulrych 1979) suggests a modest elevation also of average pressure in both types of primary hyper-

This work is supported by the Swedish Medical Research Council (Project No. 14X-00016).

REFERENCES

- ANDER, R. S. 1963 The peripheral venous system. *Handbook of physiology*, Sect. 2, Circulation, 25-199.
- W. B. 1978 Cardiovascular structural adaptation, its role in the initiation and maintenance of primary hypertension. *Clin Sci Mol Med* 55: 31-22.
- BERG, S., PALMER, E. C. & WILBORN W. 1978 Pressure-independent hypertrophy of veins and pulmonary arteries of spontaneously hypertensive rats. Characterization of function, structural and histochemical changes. *Clin Sci Mol Med* 55: 311-346.
- JULIUS, S. & ESLER, M. 1975 Nervous system in hypertension. Ch. C. Thomas, Springfield, Ill.
- KAMIYA, A., RIPPE, B. & FOLKOW, B. 1979 Volume of the capillary exchange vessels measured with gravimetric method in rat hindquarters. *Microvasc Res* 17: 142-157.
- RIPPE, B., LUNDIN, S. & FOLKOW, B. 1978 Plasma volume, blood volume and transcapillary escape rate (TER) of albumin in young spontaneously hypertensive rats (SHR) as compared with normotensive controls (NCR). *Clin Exp Hypertension* 1 (1): 39-50.
- SIMON, G. 1976 Altered venous function in hypertensive rats. *Circ Res* 38: 412-418.
- TAKESHITA, A. & MARK, A. L. 1979 Decreased venous distensibility in borderline hypertension. *Hypertension* 1: 207-206.
- ULRYCH, M. 1979 Pathogenesis of essential hypertension. *Angiology* 30: 104-116.

and intravascular volume increase could be estimated with considerable accuracy over the (for rats) physiological \bar{P}_v range about 6–20 mmHg.

Results The study has allowed deduction of average venous compliance in SHR and NCR. In each group uniform results were obtained showing an almost linear relationship over the mentioned \bar{P}_v range between increases of \bar{P}_v and intravascular volume both in SHR and NCR. Between SHR and NCR there was however a clearcut difference since the intravascular volume increase/mmHg \bar{P}_v elevation $\times 100$ g tissue was 0.101 ± 0.005 (SE) in NCR but only 0.083 ± 0.004 in SHR at complete vascular relaxation. This 20% difference tended if anything to become reduced when graded noradrenaline vasoconstrictions were induced which caused largely equal increases of \bar{P}_v and venous resistance in SHR and NCR but in SHR the characteristic structural accentuation of precapillary resistance increase (Folkow 1978). It should here be noted however that venous resistance and capacitance changes are not necessarily congruent since e.g. venular sections may contribute far more to resistance than to capacitance.

To estimate average venous distensibility also the initial absolute ('control') venous volumes must be defined at least in approximate but for NCR and SHR equivalent figures. They can only be deduced indirectly though with reasonably small error from total hindquarter intravascular volumes if corrected for precapillary and capillary volumes. Flow and mean transit time measurements gave control intravascular volumes of 5.5 ± 0.2 ml/100 g hindquarters being if anything, slightly larger in SHR than in NCR.

In most vascular beds the precapillary section contains some 70% of total contents and the hindquarter exchange vessels very nearly 10% (Kamlitz, Rippe & Folkow 1979) leaving about 70% for the venous compartment (cf. Alexander 1963). There is no reason to believe that SHR and NCR differ appreciably concerning the small capillary exchange volume but the 40–50% higher precapillary resistance in SHR (Folkow 1978) implies a 20% smaller microvascular precapillary volume though offset by usually slightly wider large arteries in SHR. Anyway with only 20% total precapillary volume a minor SHR/NCR difference hardly affects the deduced control venous volumes which would then be about 3.8 ml/100 g hind

quarter in both NCR and SHR. —As the regional \bar{P}_v elevations raise also precapillary resistance though to a lesser degree, precapillary resistance contributes to the volume increases less since the precapillary volume and pressure are much smaller and arterial walls far stiffer. About 90% of the volume increases to \bar{P}_v elevations reflect venous distension (Alexander 1964).

Conclusion and comments The combined 70% reduction of venous distension in SHR is anything a slightly larger control venous volume suggests that the difference between SHR and NCR is not merely one of unstressed hindquarter venous volume. It rather reflects a true relative hindquarter venous wall compliance, which must reflect a structural stiffening and/or thickening of the venous non-contractile wall during SHR. Expressing venous compliance as per volume increase per mmHg increment of venous pressure (\bar{P}_v) the figure for the NCR venous capacitance side is about 2.70% for SHR. Estimations of venous distension during noradrenaline vasoconstriction is difficult to quantify as venous distension is reduced but also in this situation the distensible vessels seemed to be modestly stiff. Differences in venous wall compliance are small but hemodynamically they can be important for volume distribution, cardiac filling pressures in hypertension.

In general these signs of a structural reduction of the venous capacitance in SHR hindquarters agree well with recent anatomic data from isolated SHR veins by Grollman & Wilborn (1978). However the absence of a significant venous pressure effect in the acute measurements led to the interpretation that venous wall hypertrophy may be independent in SHR. Even though also other findings suggest a genetic facilitation of structural cardiovascular adaptation in SHR (Folkow 1979) relationships between load and tissue design are judged on the basis of average pressures over periods particularly for veins. The reason for wide fluctuations in both venous pressure and neurogenic venous engagement during daily life. For example if the daily average for central venous pressure rose from 2 to 2.5 mmHg, an increase difficult to ascertain since fluctuations around the mean are much larger, this would be enough to cause 25% wall thickening. The fact the increased

formation of 15-hydroxy 5,8,11,13-eicosatetraenoic (15-HETE) as a major metabolite of arachidonic acid in human lung

AMBERG PER HEDQVIST and KJELL RÅDEGRAN

Department of Chemistry and Physiology, Karolinska Institute, Stockholm S-141 86, Sweden

Arachidonic acid in guinea pig has been shown to afford 15-HETE by a lipoxygenase pathway and to a considerably greater extent 12-HHT by the fatty acid synthetase pathway. In addition, small amounts of 11-HETE and 15-HETE (1.6 and 0.1% of total products, respectively) were isolated (Rigby & Samuelsson 1974). The corresponding arachidonic acid transformation in human is not known. However, it might be expected to differ, since chopped human lung has been shown to release PGE_2 and $\text{PGF}_{2\alpha}$ (analysis by GC or radioimmunoassay) (Piper & Walker 1976).

In a previous communication we wish to report the transformation of arachidonic acid in human lung with special reference to the pattern in asthmatic tissue. The results show an extensive conversion into C_{20} monohydroxy acids, notably 15-HETE, in asthmatic lung, and similar but less conversion in non-asthmatic lung.

Materials. Lung specimens were obtained from 6 patients of either sex undergoing pulmonary surgery. Four of the patients were non-asthmatic, and the remaining two had a long history of severe asthma (type I allergy). All patients were on the same premedication and anaesthesia, the anaesthesia in addition with terbutaline. At the time of surgery, one of the asthmatic patients showed a normal spirometric reversibility of bronchoconstriction on inhalation of isoproterenol, whilst the other asthmatic was almost symptom-free and with normal spirometric values possibly as a result of successful preoperative bronchodilation. The lung sample from the latter patient converted arachidonic acid at the same rate as those from the non-asthmatic. Samples of peripheral and macroscopically fresh lung (3-4 g) were chilled and rinsed in ice-cold

Tyrodé solution immediately after the resection. The tissue was subsequently minced and homogenized in 3 volumes of potassium phosphate buffer (0.1 M, pH 7.4) containing 10-100 μg of [^3H] arachidonic acid. The homogenates were shaken aerobically at 37°C for 30 min. Ethanol was added and the incubation mixtures were extracted twice with diethyl ether at pH 3. The residue obtained after evaporation of the solvent was treated with diazomethane and subsequently subjected to thin layer radiochromatography. The solvent systems used were the organic layer of ethyl acetate-2,2,4-trimethylpentane-water (50:100:100, v/v) (analysis of monohydroxy esters) and methanol-diethyl ether (2:98, v/v) (analysis of methyl esters of prostaglandins and TXB_2).

Results. Incubation of [^3H] arachidonic acid with 1 asthmatic and 4 non-asthmatic lungs resulted in a low (3-6%) conversion into monohydroxy acid(s) as judged by thin layer radiochromatography. The R_f value of the peak of monohydroxy acid(s) ($R_f=0.52$) was the same as the R_f values of 11-, 12- and 15-HETE, but different from that of 12-HHT ($R_f=0.45$). It thus seemed likely that the hydroxy acid peak was due to C_{20} monohydroxy acid(s) but the composition of this material was not studied further. Thin layer radiochromatographic analysis using a more polar solvent system revealed a peak of variable intensity at the origin, corresponding to phospholipid-bound [^3H] arachidonic acid. In one out of the four non-asthmatic lungs there was in addition a trace of radioactivity associated with the reference spot of PGE_2 . Formation of $\text{PGF}_{2\alpha}$, PGD_2 , 6-keto- $\text{PGF}_{2\alpha}$ or TXB_2 was not observed.

Incubation of [^3H] arachidonic acid with homogenate of the other asthmatic lung led to substantial (25%) conversion into material migrating only like

REFERENCES

- K. HEDQVIST H & SVANBORG N 1974. *ed plasma levels of 15-keto-13,14-dihydro-
glandin F_{2α} after allergen-provoked asthma in
Lancet* II 1419-1421
- G. M & SAMUELSSON B 1974 Prosta-
endoperoxides VII Novel transformation of
donic acid in guinea pig lung. *Biochem Biophys
commun* 61 942-949
- ST P DAHLÉN S-E, GUSTAFSSON
ANMARSTRÖM S & SAMUELSSON B.
→ Biological profile of leukotrienes C and D.
Physiol Scand 110 (in press)
- A. A. 1976 Studies on actions of prostaglandins
lung. *Acta Physiol Scand. Suppl* 441 1-55
- A. A., HEDQVIST P HOLMGREN A. &
→ BORG, N 1973 Bronchial hyperreactivity to
aglandin F_{2α} and histamine in patients with
in *Br Med J* 1 193-196
- MONCADA S. & VANE, J R. 1979 Pharmacology and
endogenous roles of prostaglandin endoperoxi-
des, thromboxane A₂, and prostacyclin. *Pharmacol Rev* 30:
293-331
- MONCADA S, GRYGLEWSKI R., BUNTING, S. &
VANE, J R. 1976. A lipid peroxidase inhibits the en-
zyme in blood vessels that generates from prosta-
glandin endoperoxides the substance (prostaglandin X)
which prevents platelet aggregation. *Prostaglandins*
12: 715-733
- PIPER, P J & WALKER, J L. 1973 The release of
spasmodic substances from human chopped lung
tissue and its inhibition. *Brit J Pharmacol* 47 791-804
- SAMUELSSON H BERGEAT P HAMMARSTRÖM
S & MURPHY R. C. 1980. Leukotrienes. A new
group of biologically active compounds. In: *Advances
in prostaglandin and thromboxane research* vol 8
(ed H Samuelsson H Razavet & R. Paoletti) pp
1-18. Raven Press New York

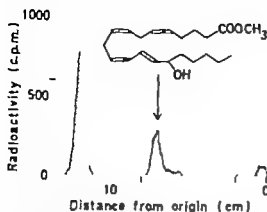


Fig. 1 Thin layer radiochromatogram of esterified product obtained following incubation (37° 30 min) of 100 µg [3 H]arachidonic acid with a whole homogenate of 1 g of asthmatic lung in 9 ml of 0.1 M potassium phosphate buffer pH 7.4. The solvent system was the organic layer of ethyl acetate–4-trimethylpentane–water 50:100:100 (v/v/v).

C_{20} monohydroxy acids ($R_1=0.5^*$) (Fig. 1). Part of the material was converted into the trimethylsilyl ether derivatives and analyzed by mass spectrometry. The mass spectrum showed ions of high intensity at m/e 406 (M), 335 ($M-71$) loss of $(CH_3)_3C-CH_2$, 316 ($M-90$ loss of trimethylsilanol), 225 $[(CH=CH-CH=CH-CH(OTMS)-(CH_2)_7-CH_3)]$ and 173 ($TMSi-O-CH-(CH_2)_7-CH_3$) indicating that the parent acid was 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE). However, the mass spectrum also showed weak ions indicating the presence of small amounts of 11-HETE and 11-HPETE. In order to determine the percentage composition of the C_{20} monohydroxy acids, the material was subjected to catalytic hydrogenation converted into the TMSi ether derivatives and analyzed by mass spectrometry. Several spectra were recorded on the hydroxy acid peak, as were spectra on the references, the TMSi derivative of methyl 11, 12- and 15-hydroxyeicosanoates. The references gave each two ions of high intensity, i.e. m/e 229 and 87 (11-hydroxy), m/e 215 and 301 (12-hydroxy) and m/e 173 and 343 (15-hydroxy). The ions mentioned were present in the sample showing the following percentage composition of hydroxy acid isomers: 15-HETE (89%), 11-HETE (9%) and 11-HPETE (2%).

Discussion PGF $_{2\alpha}$ is a potent bronchoconstrictor in particular in patients with bronchial asthma (Mathé et al. 1973) and the plasma levels of 15-keto-13,14-dihydro-PGF $_{2\alpha}$ (metabolite of PGF $_{2\alpha}$) increase markedly when such patients are provoked

with specific allergen (Green, Hedqvist & 1974). As a consequence PGF $_{2\alpha}$ has been considered to have a mediator role in bronchial asthma. The present results do not necessarily rule out this possibility, but they indicate that transfer of arachidonic acid by lipoxygenase rather than cyclooxygenase seems to be the preferred pathway in human lung parenchyma. Thus the tissue showing the highest conversion of arachidonic acid (25%) and hence offering the best chance for formation of products was found to give rise predominantly to the C_{20} monohydroxy acids 15-HETE (89%), 11-HETE (9%) and 11-HPETE (1%). While 15-HETE can be formed by the cyclooxygenase system, it may be noted that this tissue was obtained from a patient with bronchial asthma. The same radiochromatographic pattern was seen in the remaining 5 experiments, although due to a low conversion rate the material could not be fully analyzed by mass spectrometry. In one of the asthmatic lungs there was an additional peak of radioactivity having the same R_f value as 15-HETE, it is suggested therefore that 15-HETE is a metabolite of arachidonic acid in asthmatic lung.

Presently it is not known whether 15-HETE has any pulmonary effects. However, its immediate precursor 15-HPETE is a well-known inhibitor of prostacyclin synthetase (Moncada 1976). Prostacyclin is a potent vasodilator, it has been reported to reduce unspecifically the bronchoconstriction in man (cf. Moncada & 1979). Since prostacyclin is released from the lungs, presumably from vascular endothelium, in several animal species, it is at least theoretically possible that inhibition of prostacyclin formation by 15-HPETE could alter the perfusion-ventilation relationships in the human lung.

In the present study there was no indication of formation of 5-HPETE, parent substance of the more active leukotrienes (Samuelsson & 1980; Hedqvist et al. 1980). It should be kept in mind, however, that 15-HPETE and 5-HPETE are structurally very similar and that they might give rise to compounds having similar effects. Experiments are in progress to investigate this interesting possibility.

Supported by grants from the Swedish Medical Research Council (projects 04X-434 and 03X-5170) and the Karolinska Institutet.

nociceptive influence from trunk region on monosynaptic hind limb reflexes in the cat

L. SON and C. R. SKOGLUND

Dept of Physiology II, Karolinska Institute, Stockholm, Sweden

In a previous investigation (Carlsson & Lindquist 1976) it was shown that stimulation of the trunk skin nerve in eliciting reflexes in the lumbar back. Nociceptive stimulation of the dorsal aspect of the trunk evokes reflex activity in back of both sides whereas stimulation applied to skin regions located ventrally may inhibit tonic muscle activity. Effects of limb skin stimulation upon back muscle reflex activity were observed, indicating the presence of reflexes controlling the activity in trunk muscles during withdrawal movements of the limbs.

The aim of the present series of experiments was to study the possible influence of trunk skin afferents on proprioceptive reflexes of hindlimb muscles. Adult cats were used for the experiments. Anaesthesia was initiated by ether and as 4 cats were used by 1. administration of chloralose (mg per kg wt.) In 5 animals the spinal cord was exposed at the C₆ level after ligation of the 12th rib. The animals were curarized by injection of Flaxedil and ventilated artificially. Electrophysiology (L₁-L₅) was performed on 6 cats. Nerves to flexor digitorum brevis (FDB) and (FDL), gastrocnemii lateralis and medialis (GL and GM), tibialis anterior and extensor digitorum (peroneus PER) as well as skin

and ventral and dorsal root were prepared for recording and stimulation. Monosynaptic reflexes were elicited either by stimulation of dorsal root and recording from muscle nerves or by stimulation of muscle nerves and recording from dorsal roots, as indicated in Fig. 1. These test reflexes were conditioned by mechanical stimulation of the trunk skin (pinch with a pair of forceps). Electrical stimulation of different trunk skin nerves (square wave pulses of 0.1-0.4 ms duration, 10-100 Hz, up to 15 times threshold).

Effects of mechanical trunk skin stimulation on test reflexes as well as inhibitory effects on test reflexes could be demonstrated by pinch stimulation of the skin. Light touch or pressure was also used. In GM inhibition was regularly seen

when stimulating the skin covering the tail and the dorsal side of the trunk up to a level corresponding to the 3rd lumbar vertebra. The inhibitory effect was most pronounced by pinching at the lower lumbar levels and gradually decreased at higher levels. Inhibition was also observed when stimulating the contralateral skin areas. Similar effects were observed on test reflexes in FDB and FDL, supplying toe flexors in PER, which innervates ankle flexors and toe extensors, no clear effects of dorsal skin stimulation could be demonstrated. Pinch stimulation applied to ventral skin areas on the trunk did only occasionally change the test reflexes in the different muscle nerves. In some experiments however facilitation of the test reflex in PER occurred. From these observations it is concluded that trunk skin nociceptive afferents have connections with motoneurons supplying muscles acting at the ankle and in the foot.

Effects of electrical trunk skin nerve stimulation
The nervous connections between the trunk skin afferents and the limb muscle motoneurons were further studied in a series of conditioning test reflex experiments using electrical stimulation of skin nerves. Stimulation at varying intensities was applied to cutaneous nerve branches from the dorsal and ventral rami of the spinal nerves supplying the dorsal and ventral aspects of the trunk respectively.

In most of the nerves tested (GM, GL, FDB and FDL) the monosynaptic reflexes were usually inhibited by the conditioning volleys in the dorsal skin nerves (see figure). The inhibition was observed at test-conditioning intervals ranging from 15 up to 40 ms and was most prominent at the longer intervals. In PER, however facilitation was generally observed as exemplified in the uppermost pair of records. The small monosynaptic test reflex (first peak in left-hand record) showed 3-fold increase in amplitude after conditioning (second peak in right-hand record). In this experiment also a multisynaptic volley appeared which likewise showed some facilitation. The volley preceding the con-

Ischemic coma in rats at different pre-ischemic blood glucose levels Cerebral metabolic recovery after ischemia¹

JUSZ SIEMKOWICZ² and ALBERT GJEDDE³

¹Units of Anesthesia, University Hospitals (Hvidovre) and ²Medical Physiology A, University Institute, Copenhagen University, Denmark

SIEMKOWICZ, J. & GJEDDE, A. Post-ischemic coma in rat: Effect of different pre-ischemic blood glucose levels on cerebral metabolic recovery after ischemia. *Acta Physiol Scand* 1980, 110: 225-232. Received 2 Aug. 1979. ISSN 0001-6772. Departments of Anesthesia, University Hospitals (Hvidovre) and Medical Physiology A, The Panum Institute, Copenhagen University, Denmark.

Hyper-, normo- and hypoglycemic rats were exposed to 10 min of complete cerebral ischemia. Regional cerebral blood flow (CBF), blood-brain glucose transfer and cerebral consumption of oxygen and glucose were measured before, as well as three and 60 minutes after ischemia. Three min after ischemia, no differences were observed between the 3 groups of rats. One h after ischemia, the hyperglycemic rats in comparison with those of the other groups had lower whole-brain CBF and glucose consumption but appreciably lower oxygen consumption, indicating continued non-oxidative use of glucose in the hyperglycemic group. In general, regional CBF values exceeded the control value by 100-200% 3 min after ischemia and were reduced to 50% of control at 1 h after ischemia, at which time the rats were still comatose. In the brain stem of hyperglycemic rats, blood flow, however, remained elevated after ischemia. Thus, the significantly increased mortality observed in rats hyperglycemic before, during and after ischemia (Siemkiewicz & Hansen 1978) was the result, not of impaired postischemic CBF, but of ischemic or postischemic damage to brain cells. We suggest that the damaging factor in the hyperglycemic group is increased lactacidosis associated with prolonged anaerobic glycolysis.

Key words: Cerebral ischemia, cerebral blood flow, hyperglycemia, postischemic recovery

Siemkiewicz & Hansen (1978) reported the finding that high blood glucose in experimental animals led to short episodes of global cerebral ischemia associated with considerably reduced respiration when compared to animals with normal or low blood sugar. Myers & Yamaguchi (1976) ascribed the same observation with the greater lacidosis of brain tissue encountered in and after ischemia in monkeys with high blood sugar.

Ischemic damage to the brain may occur during the ischemic insult and may first affect the function of cells (the "cellular factor") (Levy et al. 1975a) or the patency of vessels (the "vascular factor") (Auer et al. 1968). Although recent studies have failed to detect gross impairments of blood flow after short episodes of brain ischemia in animals with normal blood sugar (Ginsberg et al. 1975b), it was possible that perfusion impairments intervened in the immediate post-

ischemic period of hyperglycemic animals. Therefore the present study was intended to reveal post-ischemic differences of cerebral blood flow or metabolic rates for oxygen and glucose between groups of rats with low, high or normal blood glucose before a well-defined episode of complete cerebral ischemia lasting 10 min.

MATERIALS AND METHODS

The brains of normo-, hyper- or hypoglycemic rats were rendered ischemic for 10 min. Gross regional cerebral blood flow and blood-brain glucose transfer rates, as well as whole-brain metabolic rates for oxygen and glucose, were determined by the method of Gjeddé et al. (1980).

Presented in part at the 101st meeting of the American Neurological Association, Washington, D.C., September 1978 and at the Symposium on Cerebral Metabolism and Neural Function Bethesda, Md., May 1979.



Fig. 1. Facilitation and inhibition of hind limb reflexes by conditioning volleys in trunk skin nerves. Left column: unconditioned test reflexes elicited (STIM) and recorded (REC) as indicated. Dr: dorsal root. Vr: ventral root. Right column: test reflexes after conditioning volleys in skin nerves at different lumbar levels (LEV). Conditioning test intervals (INT) in ms. See text.

ditioned monosynaptic response represents a reflex response elicited by the conditioning stimulus itself as evidenced by other experiments. These reflex responses in PER indicate the presence of excitatory connections between the dorsal trunk skin and motoneurons supplying ankle flexors and/or toe extensors. Reflex discharges caused by the conditioning volley alone were never observed in any other limb nerve tested. Inhibitory as well as facilitatory effects were elicited at stimulus strengths evoking reflex activity in the back muscles. Weaker stimulation was ineffective and higher intensity did not change the general nature of these effects. Conditioning stimulation delivered to dorsal skin nerves at other segmental levels (L_7-L_6) and to corresponding contralateral nerves had similar effects on the different test reflexes as described above.

The reflex effects of stimulation applied to ventral skin nerves proved to be less marked than those originating from the dorsal skin nerves and displayed a higher degree of variability from one preparation to another. A common pattern found was a

slight facilitation of the test reflexes at conditioning intervals between 70 and 40 ms. On several occasions no signs of facilitation were observed. Thus, following ventral skin nerve stimulation no distinct difference flex effects on motoneurons supplying toe extensors in the lower limb and in the foot established (cf. dorsal skin nerve stimulation above).

The results from the test-conditioning experiments suggest that there are special mechanisms for facilitation and inhibition of limb motor output from trunk skin. The latencies at which effects occur suggest reflex interaction through polysynaptic chains of interneurons. There is evidence for the presence of reflexes over several segmental levels ipsilaterally and contralaterally. Some degree of reciprocity between dorsal and ventral trunk skin seems to be present but a prominent feature of trunk skin stimulation is inhibition of the ankle extensors in both hind limbs. Functionally in a standing position, this may promote the lowering of the body towards the ground in order to avoid a stimulation applied to the dorsal side of the trunk. In this respect the effects described can be regarded as part of withdrawal mechanisms.

The results may have some bearing on findings in man of suppression of leg reflexes in painful states in the lumbar back region. In disc prolapses a depressed ankle or foot reflex may be due to blocking of nerve fibres in the roots. In cases where no herniation is present depression of reflexes nevertheless is present. Instead of a functional inhibition from pain afferents a motoneuron pool has been suggested (Morrison & Robertson 1976). Our present results provide experimental evidence for the existence of such a mechanism in the cat.

This work was supported by the funds of Kungliga Institutet Sventiska Sällskapet för Medicinsk Forskning, Magn Bergwall's Stiftelse.

REFERENCES

- CARLSON H & LINDQUIST C. 1976. Evidence for influence on the lumbar back muscle tone in the cat. *Acta Physiologica Scandinavica* 97: 331-343.
MOONEY V & ROBERTSON J. 1976. The lumbar dromy. *Clin Orthopaedics* 115: 149-157.

Physiological variables of rats included in the present study

expressed as mean \pm S.E. Numbers in brackets indicate animals in which only arteriovenous deficits were

	Time after ischemia	Group		
		Normal glucose	High glucose	Low glucose
no. of animals	Control	5	4	4
	3 min	6 (3)	6 (3)	5 (3)
	60 min	6	8	7
aHg)	Control	130 \pm 13	145 \pm 20	142 \pm 20
	3 min	142 \pm 10	148 \pm 7	128 \pm 5
	60 min	142 \pm 11	157 \pm 6	155 \pm 5
mmHg)	Control	33 \pm 2	37 \pm 0.4	35 \pm 2
	3 min	37 \pm 2	33 \pm 2	35 \pm 2
	60 min	32 \pm 4	35 \pm 3	32 \pm 0.5
	Control	7.47 \pm 0.02	7.41 \pm 0.03	7.39 \pm 0.02
	3 min	7.36 \pm 0.04	7.31 \pm 0.04	7.28 \pm 0.02
	60 min	7.45 \pm 0.05	7.32 \pm 0.02	7.37 \pm 0.03
(mmHg)	Control	105 \pm 3	103 \pm 13	101 \pm 8
	3 min	124 \pm 7	158 \pm 16	146 \pm 1
	60 min	109 \pm 5	111 \pm 7	93 \pm 9

and 3 min after the termination of ischemia all arteriovenous deficits could not be carried out as easily. Consequently in these experiments measurements of glucose were made in one group of animals and the measurements of flow etc. in another. No variables in these two groups differed significantly with respect to the arterial plasma glucose concentration as higher in the groups in which the superior arterial system was cannulated after ischemia (cf. Table 2) glucose consumption (CMR_{glu}) was calculated from an arterial deficit of one group and the cerebral blood flow of another, while the "arteriovenous" glucose flux was calculated from values observed in the same rat. Absolute values for CMR_{glu} and J^* are not com-

pared with those of rats containing 1.5 ml of Soluene (Packard) and isopropenol mixture (11). The vials and samples were then reweighed, bleached with 0.5 ml 35% hydrogen peroxide and stored overnight at 40°C. The next morning, preparation was completed by addition of 20 μ l of an isotope (Packard) and 0.5 N HCl mixture (9, 11). The vials were allowed to stabilize at counting temperature for 24 h prior to counting in Packard Tri-Carb 425 liquid scintillation spectrometer.

Preparations of α -[1- 14 C]glucose, D-[1- 3 H]glucose and [3 H]deoxyglucose were obtained from New England Nuclear Corporation with specific activities of 1.36 mCi/mmol, 18 Ci/mmol and 318 mCi/g 14 C respectively.

At the time of the experiment the animals were in respiratory steady-state with 5 μ Ci [14 C]glucose and 20 μ Ci [3 H]glucose in 1 ml Ringer-HEPES buffer solution (Oxoid & Cross) rapidly injected into the carotid vein. Prior to this, the [3 H]glucose solution was air-dried and resuspended in phosphate saline H $_2$ O. Integrated concentration-time curves of arterial blood produced mechanically as described previously (de Groot 1983). Briefly one or two arterial samples drawn into calibrated glass syringes at known and set rate during and for 30 s after injection. At the end of the experiment the cannula was disconnected and the animal decapitated with a rodent guillotine. The brain was rapidly cooled and divided bilaterally into the following regions, weighing from 30 to 100 mg each: cerebellar hemisphere, olivary nucleus, medulla and pons, midbrain, basal ganglia, parietal cortex and frontal, occipital, and temporal lobes. The remainder of the brain was counted as one. Arterial and tissue samples were rapidly transferred

RESULTS

Arterial tensions of oxygen and carbon dioxide, arterial pH and mean arterial blood pressure were monitored in all rats and met the following criteria established for inclusion in the present study: P_aO_2 \geq 100 mmHg, $30 < P_aCO_2 < 40$ mmHg and MABP \geq 100 mmHg before and 3 min after the end of ischemia (Table 1). Arterial concentrations of glucose and oxygen varied as shown in Table 2.

Cerebral blood flow during ischemia

Gross regional blood flow rates were measured in two rats 5 min after the onset of ischemia (Table 3). The flow rates were uniformly below 1% of the normal rate.

consisting of an i.v. bolus administration of a mixture of labeled butanol and labeled D-glucose with subsequent determination of the brain content of these tracers. Whole brain metabolic rates for glucose and O₂ were calculated from the product of cerebral arterio-venous deficits and whole-brain blood flow rates.

Ischemic model

The production of 10 min of cerebral ischemia in the rat has been described in detail elsewhere (Siemkowiec & Hansen 1978). However, absolute values of blood flow during ischemia were not assessed previously.

Following ether induction, the rats were intubated with steel tubes. Three min prior to ischemia, all animal received 50 IU/kg heparin sodium i.v. and halothane administration was discontinued. Between 1 min before and 1 min after the onset of ischemia, arterial blood was slowly removed until the mean arterial blood pressure reached 50 mmHg during ischemia. Cerebral ischemia was introduced by inflation of a pneumatic cuff around the neck to a pressure of one atmosphere.

After 10 min of ischemia, the cuff was rapidly released and the arterial blood reinfused in 30–45 s. To the last ml of blood, 5 µg adrenaline was added, causing the mean arterial blood pressure to rise to 150–180 mmHg. All rat treated with insulin received an i.p. injection of 40% glucose solution at the end of ischemia in order to raise plasma glucose to levels comparable with those of the control group in the postischemic period.

Determination of cerebral blood flow and blood-brain glucose transfer rate

Whole brain and regional cerebral blood flow rates were calculated from tissue and arterial blood sample contents of labeled butanol 70 s (= T) after an i.v. injection using an equation relating cerebral blood flow per unit weight of brain (F^{br}), the amount of butanol present in unit weight of brain (C_{br}(T)) and the amount of butanol present in an arterial sample (Q_a(T)) collected at a constant and known rate (Fⁱⁿ):

$$F^{br} = \frac{1}{E(T)} F^{in} \frac{C_{br}(T)}{Q_a(T)} \quad (1)$$

where E(T) is the net extraction fraction in the time T.

Whole brain and regional unidirectional blood-brain glucose transfer rates were calculated from extravascular brain tissue and arterial blood sample contents of labeled glucose assuming no loss of labeled glucose from the brain during the experiment:

$$J = F^{in} F^{br} \frac{C_{ex}(T)}{Q_a(T)} \quad (2)$$

where J represents the unidirectional glucose transfer rate per unit weight of brain, C_a^{pl} the arterial plasma glucose concentration, Fⁱⁿ the plasma flow into the sampling syringe, C_{ex}(T) the extravascular content of labeled glucose in unit weight of brain and Q_a(T) the content of labeled glucose in the arterial sample.

Whole brain values were obtained by adding from regional samples and left-over brain tissue fraction of extracted tracer glucose was calculated from the equation:

$$E_{br} = \frac{J}{C_a^{pl} F^{in}}$$

E(T) for butanol was estimated from the rate of butanol from the brain and the rate of decay of the concentration of labeled butanol. Since E(T) varied with the cerebral blood flow as explained previously (Gjeldre et al. 1980), equation (1) was solved by an iterative procedure.

The rate of decay of the monoexponential concentration curve for butanol, as well as the plasma labeled glucose placed intravascularly in rats at the time of decapitation, were calculated from the constant labeled butanol and glucose in arterial blood at the time of decapitation. The volume of plasma in brain was determined from labeled dextran in separate experiments. In these experiments, rats were injected with [11C] dextran (molar weight 70,000) and the 11C-activity per unit weight of brain volume of arterial plasma determined at the time of decapitation after 3 min circulation.

General procedure

Male Wistar rats were divided in 3 groups. In the normal glucose group received no pretreatment. In the low glucose group received 10 kg insulin (Leo) i.p. 1 h prior to study. Rats in the high plasma glucose group received 17 mmol/kg D-glucose i.p. 1 h prior to study.

The 300–350 g rats were kept specific-pathogen-free, were starved overnight. Anaesthesia was induced with ether. The animals were paralyzed with 3 mg suxamethonium and were intubated and equilibrated with 1% halothane vaporized in a mixture of 50% oxygen (70–30).

Catheters were placed in the tail artery, both femoral arteries, one femoral vein, and in most rats, also below the superior sagittal sinus of the brain. Care of the sinus was performed as described previously (Gjeldre et al. 1980).

Arterial and cerebral venous blood samples were drawn for pO₂ and P₅₀ with microdialysis (Radrometer). Arterial hematocrit was measured by centrifugation. Plasma concentrations of glucose were determined in freshly sampled arterial and cerebral venous plasma by the glucose oxidase method (Christensen, Hjelm & De Verdier 1963). Arterial and cerebral concentrations of oxygen were determined after carbon monoxide displacement in a galvanic fuel cell (Lexington Instruments). The animals were allowed to be in respiratory steady-state when PaO₂ samples taken at least 10 mm apart agreed within 10%. Arterial blood pressure was continuously recorded with a gauge transducer.

Since metabolic rates were calculated by multiplying of arteriovenous deficits with blood (oxygen or glucose) flows in brain, care was taken to draw the samples for determination of arteriovenous deficits as before flow measurements as possible. In the

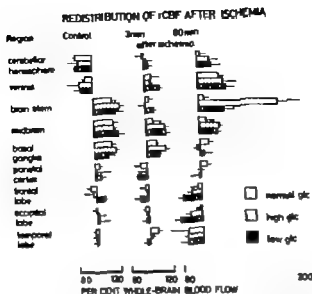


Fig. 1 Regional distribution of cerebral blood flow rates in rats with different plasma glucose levels in control condition and 3 and 60 min after cerebral ischemia of 10 min duration. Each bar represents per cent of whole-brain blood flow (\pm S.E.) at respective conditions.

ol state while at 60 min, the cerebral blood flow was higher in infratentorial regions than in the supratentorial regions. However, no significant differences in distribution were observed between the groups of rats with the exception of the brain stem

of rats pretreated with glucose in which an increase to 175% of the whole-brain average was seen 60 min after ischemia, indicating no post-ischemic reduction of absolute blood flow in this region.

The selective reduction of the blood flow of sup-

Fig. 4 Whole-brain blood flow, metabolic rate for oxygen and glucose and the oxygen-glucose index are recorded after 10 min of complete cerebral ischemia. Data are expressed as mean \pm S.E.

Variable	Time after ischemia	Group		
		Normal glucose	High glucose	Low glucose
\dot{Q} (ml (100g) min ⁻¹)	Control	129 \pm 8	87 \pm 20	116 \pm 13
	3 min	168 \pm 28	192 \pm 38	299 \pm 52*
	60 min	65 \pm 6*	64 \pm 11	46 \pm 4*
R_{O_2} (μ mol (100g) min ⁻¹)	Control	379 \pm 49	433 \pm 76	313 \pm 63
	3 min	179 \pm 58*	205 \pm 71*	321 \pm 116
	60 min	196 \pm 18*	94 \pm 9*	174 \pm 22
R_{glc} (μ mol (100g) min ⁻¹)	Control	67 \pm 10	74 \pm 6	33 \pm 4*
	3 min	111 \pm 35	118 \pm 40	187 \pm 64
	60 min	41 \pm 12*	38 \pm 11	27 \pm 7*
Oxy. glucose index (%)	Control	95	97	158
	3 min	27	28	28
	60 min	80	42	107

* significantly different from pre-ischemic value at $P < 0.05$ or less.

* significantly different from value at 3 min postischemia at $P < 0.05$ or less.

* significantly different from mean value of the other groups at $P < 0.05$ or less.

Table 2 Arterial concentration of glucose (plasma) and oxygen (blood) before and after ischemia. Values are expressed as mean \pm S.E. Numbers in brackets indicate animals in which only arteriovenous differences were measured

Variable	Time after ischemia	Group		
		Normal glucose	High glucose	Low glucose
glc (mM)	Control	10.4 \pm 0.9	10.8 \pm 0.7	1.8 \pm 0.6
	3 min	11.1 \pm 1.1	76.3 \pm 5	7.3 \pm 1.6
	60 min	(14.1 \pm 1.1)	(15.5 \pm 4)	(13.9 \pm 1.3)
O ₂ (mM)	Control	9.1 \pm 0.1	10.4 \pm 0.5	9.1 \pm 0.7
	3 min	9.4 \pm 0.3	10.3 \pm 0.1	9.7 \pm 0.3
	60 min	9.8 \pm 0.3	10.0 \pm 0.5	10.4 \pm 0.3

Cerebral blood flow and metabolic rate before ischemia

The average whole brain blood flow rates and the metabolic rates for oxygen and glucose of the three groups before ischemia are shown in Table 4. Glucose consumption was insignificantly increased in the high glucose group and markedly reduced in the low glucose group.

Oxygen consumption was similar in the three groups. The oxygen glucose index (OGI) was near 100% ($\text{OGI} = \text{CMR}_{\text{O}_2} / 100 / \text{CMR}_{\text{glc}} \times 6$) in the normal and high glucose groups before ischemia. In the low glucose group, on the other hand, oxygen consumption exceeded the rate calculated stoichiometrically from glucose consumption, indicating metabolic use of other substrates than glucose, as also noted by Norberg & Siejko (1976).

Table 3 Cerebral blood flow during ischemia

Region	CBF (ml (100 g) min ⁻¹)
Cerebellar hemisphere	0.2
Vermis	0.1
Pons	0.7
Midbrain	0.4
Basal ganglia	0.2
Parietal cortex	0.1
Frontal lobe	0.05
Occipital lobe	0.03
Temporal lobe	0.1
Cervical cord	11.4
P _{ao} (mmHg)	118
P _a (mmHg)	33
pH	7.51
MABP (mmHg)	50

Mean of estimates at 5 min of ischemia.

Cerebral blood flow and metabolic rate after ischemia

Three min after the end of the ischemia, whole brain blood flow was increased in all groups of rats. No significant differences were observed between the groups (Table 4). The rate for oxygen in the normal and high glucose groups was decreased to about 60% of control, unaltered in the low glucose group. The rate for glucose was slightly increased in the normal and high glucose groups and greatly reduced in the low glucose group in which blood glucose had been raised to normoglycemic level just at the end of the ischemic episode. The OGI is approximately 30% in all 3 groups, indicating that only one third of the glucose used by the brain was oxidized.

Sixty min after ischemia, whole-brain blood flow was about 50% of the control value in all 3 groups. The oxygen consumption was lower than in all rats, but significantly lower in the high glucose group than in the other groups (Table 4). The metabolic rate for glucose was reduced in all groups.

Sixty min after ischemia, the OGI was near 100% in the normal and low glucose groups. In the high glucose group, however, the animals continued to use glucose non-oxidatively. Thus the high glucose group could be identified as the group with the lowest rate of oxygen consumption and considerable non-oxidative use of glucose (about 60%) at the end of ischemia.

The regional distribution of cerebral blood flow rates is shown in Fig. 1. Three minutes after ischemia the distribution was essentially as in

REDISTRIBUTION OF REGIONAL BLOOD-BRAIN GLUCOSE FLOW AFTER ISCHEMIA

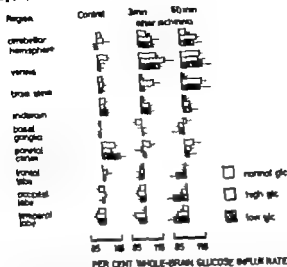


Fig. 2. Regional distribution of unidirectional blood-brain glucose flow in rats with different plasma glucose levels in control condition and 3 and 60 min after cerebral ischemia of 10 min duration. Each bar represents per cent of whole-brain glucose influx rate (\pm S.E.) at respective conditions.

marked unevenness of the distribution of the viability. Third, the favorable CBF/CMRO₂ speak against any flow-induced oxygen short in the high glucose group at 1 h after ischemia, ratio was even higher than normal (0.7). Thus, the high mortality following ischemia in hyperglycemic rats was not caused by insufficiency of blood flow. It must be related to the delayed metabolic depression (fall in CMRO₂) found in all groups after ischemia and observed to be particularly pronounced in the group of highest mortality, i.e. hyperglycemic rats. The metabolic depression in the high glucose group at 1 h after ischemia may represent inhibition of oxidative enzymes caused by the severe tissue acidosis. Low metabolic rate shortly after ischemia has been observed by several investigators (Bergh et al. 1978; Snyder et al. 1975; Steen et al. 1978). Its presence, however, is not a simple consequence of reduced functional state (e.g. trans-synaptic failure) of the brain after ischemia (post-ischemic coma) since the rats of the three groups were equally unresponsive immediately after ischemia. The delayed metabolic depression of the high glucose group after ischemia strongly suggests that the metabolism may be influenced by factors

peculiar to hyperglycemic ischemia, e.g. Lactacidosis.

What is known of the metabolic events after hyperglycemic ischemia that may point to a cause of the inefficient oxidation of glucose? The fundamental metabolic events of the immediate post-ischemic period appear to mirror those of ischemia itself. First, restoration of blood flow and oxygen delivery triggers the resumption of mitochondrial function. Inhibition of phosphofructokinase activity by increased hydrogen ion concentration (Siesjö 1978) ensures that lactate is reconverted to pyruvate before glycolysis is resumed. Labile phosphates accumulate and the (Na⁺-K⁺) ATPase proceeds to normalize extracellular ion concentrations and volume.

The duration of these events after short-lasting ischemia is known. Lactate begins to fall and labile phosphates reach preischemic levels within 2 min (Drewes & Gilboe 1973; Ljunggren et al. 1974) and the extracellular ion concentrations and volume return to normal in 3–4 min (Sienkiewicz & Hansen 1979). Lactate continues to fall for a period of time determined by its end-ischemic concentration (Ljunggren et al. 1974).

From studies of the accumulation of labile phos-

Table 5 Cerebral plasma volume and unidirectional blood-brain transfer of glucose

Values are expressed as mean \pm S.E.

Variable	Time after ischemia	Group		
		Normal glucose	High glucose	Low glucose
Whole-brain plasma volume (ml/100 g)	Control	0.88 \pm 0.08	0.82 \pm 0.18	0.79 \pm 0.06
	3 min	1.5 \pm 0.35	1.06 \pm 0.22	1.07 \pm 0.15
	60 min	0.91 \pm 0.17	0.80 \pm 0.17	0.83 \pm 0.19
Unidirectional blood-brain glucose transfer (μ mol/100 g min)	Control	137 \pm 10	174 \pm 19	121 \pm 1
	3 min	70 \pm 30	191 \pm 18	157 \pm 13
	60 min	86 \pm 8	149 \pm 29	113 \pm 16
Plasma extraction fraction (ratio)	Control	0.19 \pm 0.01	0.15 \pm 0.03	0.37 \pm 0.01
	3 min	0.18 \pm 0.02	0.09 \pm 0.01	0.14 \pm 0.02
	60 min	0.30 \pm 0.02	0.17 \pm 0.02	0.28 \pm 0.06
Transfer constant (k) (ml g ⁻¹ min ⁻¹)	Control	0.13	0.046	0.25
	3 min	0.143	0.074	0.16
	60 min	0.087	0.045	0.079

retentorial regions in favor of infratentorial regions 60 min after ischemia resembles the response seen in metabolic coma.

Blood-brain glucose transfer

The unidirectional transport of glucose from blood to brain and the extraction fraction of tracer glucose are recorded in Table 5. Regional unidirectional blood-brain glucose transfer rates are summarized in Fig. 7.

Because glucose is transported from blood to brain by a saturable carrier mechanism, it was of interest to calculate the change of blood-brain glucose transport after ischemia in the 3 groups of rats.

In all rats, the change of glucose transfer was positively correlated to the change of cerebral blood flow (Tables 4 and 5).

The unidirectional glucose transport rate has been expressed by Sokoloff et al. (1977) as a transfer constant k , multiplied by the concentration of glucose in arterial plasma. The values of k , calculated by dividing the unidirectional glucose flux by the arterial plasma concentration, are shown in Table 5. It is evident that k , the constancy of which forms the basis of the calculation of cerebral glucose consumption with 2-deoxy-D-glucose (Sokoloff et al. 1977), varies markedly.

DISCUSSION

We investigated the mechanisms underlying the observation made previously by Siemkowicz & Jensen (1978) that hyperglycemia reduces the CBF of rats after 10 min of complete cerebral ischemia.

No differences of whole-brain or regional (brain stem) blood flow distinguished the rats subjected to hyperglycemic ischemia from rats subjected to normo- or hypoglycemic ischemia, although in other respects differences were observed in the course of the study. These differences developed in the first hour after ischemia and were present at 3 min. The differences included increased cerebral oxygen consumption, greater relative use of glucose, and greater brain stem blood flow.

It was our intention to relate CBF to the metabolic demands after ischemia. Although we did not measure blood flow in regions weighing more than 30–100 mg, the following observations confirm the assumption that insufficient postischemic blood flow played no role in the development of postischemic encephalopathy:

First, the hyperglycemic rats had a higher CBF and blood flow relative to the preischemic value than the other groups at 1 h after ischemia. Second, the high unidirectional blood glucose transfer rate (Table 5) compared to the low rate in the hyperglycemic

Endometrial cholinergic secretory responses during estrous cycle pregnancy and after estrogen and/or progesterone treatment of the guinea-pig

MARETA HAMMARSTRÖM

Department of Physiology, Karolinska Institute, Stockholm, Sweden

HAMMARSTRÖM M. Endometrial cholinergic secretory responses during estrous cycle pregnancy and after estrogen and/or progesterone treatment of the guinea-pig. *Acta Physiol Scand* 1980, 110: 233-240. Received 27 Nov 1979. ISSN 0001-6772. Department of Physiology, Karolinska Institute, Stockholm, Sweden.

During estrous cycle as well as during pregnancy and after treatment with estrogen and/or progesterone uterine mucous secretory responses were studied as amount of carbohydrate released from everted guinea-pig uteri after transmural nerve stimulation or carbachol administration. In estrous cycle both stimuli increased secretion by about 20% except for the late diestrous phase, when no secretory responses were seen. In pregnancy secretory responses were noted at implantation time. From mid pregnancy and onwards secretory responses of increasing magnitude were obtained. Secretory responses persisted post partum. Evidence of an adrenergic inhibitory influence on the neurogenic cholinergic responses during pregnancy was noted. Both stimuli increased carbohydrate release by about 50% above resting level in uteri from caesarean receiving combined hormone treatment. In uteri of animals receiving estrogen only the increase was about 20%. No definite secretory response was obtained in uteri from animals receiving progesterone only. It is suggested that both hormones are necessary for full cholinergic secretory response to nerve stimulation or muscarinic drugs. It is concluded that muscarinic stimulation of the endometrium results in mucous secretion during all crucial events during the reproductive cycle. Furthermore a cholinergic secretory innervation seems to be present at all these times.

Key word: Uterus endometrium, cholinergic nerves, mucous secretion, estrous cycle, pregnancy estrogen, progesterone

previous report (Hammarström & Sjöstrand 1974) evidence of a cholinergic, presumably synaptic transmission, innervation of the guinea-pig myometrium was presented. The work presented in this paper is based on experiments on uterine gland into artificial secretory phase by a combination of estrogen and progesterone treatment. Mucous secretion was calculated from amounts of carbohydrate released from everted uteri placed in a bath.

Endometrial function is dependent on female sex hormones (vide e.g. Haschmann & Adler 1908, ex. Hertig & Rock 1950, Thomsen & Schänke 1969, Dallenbach-Hellweg 1969, Laws 1973). Exogenous female sex hormones as well as pregnancy have dramatic and diverse effects on myometrial contraction (cf. Sjöberg 1967, Öwman, Sjöberg &

Sjöstrand 1974, Thorbert 1978) as well as on the myometrium (e.g. Marshall 1970).

Thus a natural follow-up seemed to be to investigate secretory responses to field stimulation of intramural nerves and to a muscarinic drug, i.e. carbachol during estrous cycle of virgin female guinea-pigs and during pregnancy as well as post partum, i.e. when ovulation and repeated fertilization ordinarily occurs in the guinea-pig. It also appeared to be of interest to investigate secretory responses of uteri dominated by excess of estrogen or progesterone.

MATERIAL AND METHODS

Animals. Mated or albino virgin guinea-pigs (400-500 g) were used. For the study on the reproductive cycle the

phates (Ljunggren et al 1974) and the restitution of extracellular ion concentrations (Siemkowiec & Hansen 1979) it is known that these metabolic events proceed at the same rate after hyper and normoglycemic ischemia. Therefore the only characteristic which distinguishes the hyperglycemic ischemic recovery from normo- and hypoglycemic recovery is a lingering lactacidosis.

Our results indicate that circulatory insufficiency after ischemia does not distinguish the high glucose group from the other groups and that the deleterious effect of glucose pretreatment on the revival of rats after cerebral ischemia may involve abnormalities of oxidative metabolism. This conclusion is supported by the failure of Welsh et al (1978) to find any correlation between regional cerebral blood flow and deranged metabolite concentrations after ischemia in hyperglycemic animals.

This study was supported by grants from the Danish Medical Research Council (no 51-8173) and Lönberg-Madsen's Foundation. The authors wish to thank Ellen Munch and Susanne Korsholm for expert technical assistance and Berit Ree and Hildegard Mosthaf for careful typing of the manuscript.

REFERENCES

- AMES A III, WRIGHT R L, KOWADA M, THURSTON J M & MAJNO G 1968. Cerebral ischemia. II. The no-reflow phenomenon. *Amer J Pathol* 57: 437-453.
- CHRISTENSEN N J 1967. Note on the glucose oxidase method. *Scand J Clin Lab Invest* 19: 379-384.
- DREWES L R & GILBOE D D 1973. Glycolysis and the permeation of glucose and lactate in the isolated perfused dog brain during anoxia and postanoxia recovery. *J Biol Chem* 248: 489-496.
- GINSBERG M D, BUDD A W & WELSH F A 1978. Diffuse cerebral ischemia in the cat. I. Local blood flow during severe ischemia and recirculation. *Ann Neurol* 3: 482-492.
- GJEDDE A, HANSEN A J & SIEMKOWICZ E 1980. Rapid simultaneous determination of regional blood flow and blood brain glucose transfer in brain of rat. *Acta Physiol Scand* 108: 3: 1-330.
- GJEDDE A & CRONE C 1975. Induction processes in blood-brain transfer of ketone bodies during starvation. *Am J Physiol* 229: 1165-1169.
- HJELM M & DE VERDIER, C-H 1963. A logical study of the enzymatic determination of lactic acid in blood. *Scand J Clin Lab Invest* 15: 41-50.
- LEVY D E, BRIERLEY J B & PLUM F J 1974. Ischaemic brain damage in the gerbil in the absence of no-reflow. *J Neurol Neurosurg* 38: 1197-1205.
- LEVY D E, BRIERLEY J B & SILVERMAN D 1975b. Brief hypoxia-ischemia initially disrupts brain neurons. *Arch Neurol* 32: 490-494.
- LJUNGREN B, NORBERG K & SIESJÖ B 1974. Influence of tissue acidosis upon cerebral energy metabolism following total arterial occlusion. *Brain Res* 77: 173-186.
- MYERS R E & YAMAGUCHI M 1974. H. serum glucose concentration on brain respiratory arrest. *J Neuropathol Exp Neurol* 23: 10.
- NORBERG K & SIESJÖ B K 1976. Oxidative metabolism of the cerebral cortex of the rat in insulin-induced hypoglycemia. *J Neurochem* 27: 352.
- SIEMKOWICZ, E. & HANSEN A J 1979. Cerebral restitution following cerebral ischemia in hyper- and hypoglycemic rats. *Acta Neurol Scand* 58: 1-8.
- SIEMKOWICZ, E. & HANSEN A J 1980. Extracellular H⁺ and H₂O concentrations in rat brain during ten minutes of cerebral ischemia. *Acta Neurol Scand* 108: 29 A.
- SIESJÖ B K 1978. Brain energy metabolism. New York.
- SNYDER J U, NEMOTO E, M CARROLL J, SAFAR P 1975. Global ischemia in dogs: intracranial pressures, brain blood flow, and metabolism. *J Neurosurg* 42: 6: 21-27.
- SOKOLOFF L, REIVICH M, KENNEDY C, ROSIERS M H, PATLAK C S, PETTO K D, SAKURADA O & SHINOHARA M 1976. The [¹⁴C] deoxyglucose method for the measurement of local cerebral glucose utilization: Theory and normal values in the conscious and anesthetized albino rat. *J Neurochem* 28: 897-916.
- STEEN F A, MILDE J H & MICHENFELDE O 1978. Cerebral metabolic and vascular effects of barbiturate therapy following complete global ischemia. *J Neurochem* 31: 1317-1324.
- WELSH F A, GINSBERG M D, RIEDEL J, BUDD W W 1978. Impairment of metabolic recovery from cerebral ischemia by prior administration of glucose. *Fed Proc* 37: 873.

Weight endometrial carbohydrate concentration and resting secretion level of uterine gland correlated with estrogen and/or progesterone

S.D. number of experiments within brackets. Concerning uterine weight, there were statistically significant differences between the groups. No such differences were seen with respect to the endometrial carbohydrate concentration and resting secretion level of combined treatment group was lower ($P < 0.01$) than that of the two other groups

	Uterine wet weight (g)	Endometrial carbohydrate concentration ($\mu\text{mol/g}$)	Resting secretion level ($\mu\text{mol/10 ml}$)
Control and progesterone treatment	0.73 ± 0.25 (25)	44.16 (19)	0.47 ± 0.17 (21)
Control and progesterone treatment	0.93 ± 0.40 (30)	39.14 (21)	0.37 ± 0.35 (20)
Control and progesterone treatment	0.46 ± 0.14 (14)	42.26 (10)	0.60 ± 0.23 (12)
Control treatment versus estrogen and progesterone treatment	$P < 0.05$		$(P < 0.01)$
Control treatment versus progesterone treatment	$P < 0.001$	n.s.	n.s.
Control treatment versus progesterone treatment	$P < 0.001$	n.s.	n.s.

1. After shaking the homogenate was centrifuged at 1400 g for 10 min. The supernatant was removed and to it 10% trichloroacetic acid was added. After centrifugation the supernatant was removed and the carbohydrate content was determined using an external standard (vide Hammarström & Lund 1979).

2. Electrolysis was applied at standard rate of 0.5 mA/cm², 30 V which had been found to give optimal results (vide Hammarström & Spöck 1979). At every day 16–18, 23–25, 30–32, the uterine glands were excised. The placenta and fetuses were expelled. It was made it impossible to obtain an adequate spread of the electrodes through the trypsin on transvaginal stimulation. Therefore field stimulation experiments were omitted on days 16–18.

3. Apart from the homogenates obtained from Schering Carboxycholesterol chloride (Cartachol) (Sigma), Lactase hydrochloride (Sigma), phenolanthrone (Ciba), mercuric iodine hydrochloride (BDH), and Ferrous (Ferroval) were used.

4. The Student's *t*-test or when necessary the Mann-Whitney *U*-test (vide Remington & Schork 1970) were used for statistical comparison between the groups.

A detailed description for composition see Hammarström & Lund (1979).

RESULTS AND COMMENTS

Endometrial carbohydrate concentration (Figs 1 and 3)

Throughout the estrous cycle and after hormone treatment large variations in mucosal carbohydrate concentration was seen. The similarity in mucosal carbohydrate concentration is probably explained by the fact that female sex hormones alone or in combination change the relative distribution and

type of polysaccharides in epithelial glandular or stromal tissues rather than affect total carbohydrate concentration as such (cf. Schmidt-Matthiesen 1963).

Summary during pregnancy mucosal carbohydrate concentration was rather uniform. Maximum concentration was noticed in early pregnancy (day 16–18).

Resting carbohydrate secretion level (Tables 1, 2 and 3)

During the estrous cycle no statistically significant variation in resting secretion level was noted. In pregnancy maximum secretion level was noted at day 16–18, i.e. when mucosal carbohydrate concentration was largest (see above). A second maximum was noted at day 63–68 of pregnancy. No significant difference in resting secretion level between the groups receiving single hormone treatment was noted. However the group receiving combined treatment had lower resting secretion level.

Field stimulation of nerve (Figs. 1 and 2)

Estrous cycle (Fig. 1A.) A secretory response to field stimulation was elicited at the days around ovulation, i.e. estrus (day 16–18) (+20%). On day 3–5 (metestrus) field stimulation caused an inhibition of carbohydrate secretion (–15%). The discrepancy in effect on carbohydrate secretion caused by field stimulation and cartachol (see below) respectively together with previous results that possibly

Table 1 Endometrial carbohydrate concentration and resting secretion level during the estrous cycle

There was no statistically significant difference between the groups. Mean \pm S.D. number of experiments within bracket.

Day in estrous cycle	Endometrial carbohydrate concentration ($\mu\text{mol/g}$)	Resting secretion level ($\mu\text{mol}/10 \text{ min}$)
16-18	50 ± 18 (18)	0.74 ± 0.4 (1)
3-5	43 ± 16 (5)	0.71 ± 0.3 (18)
6-7	49 ± 16 (14)	0.61 ± 0.36 (13)
8-11	51 ± 17 (13)	0.85 ± 0.7 (14)
1-15	43 ± 8 (1)	0.75 ± 0.36 (10)

animals were controlled daily concerning the status of the vaginal closure membrane. Only animals showing regular cycles were used.

Animals used for the study of hormone effects were oophorectomized under sodium pentobarbital anesthesia (30-40 mg/kg) (when necessary supplemented with ether).

Determination of oestrus in the estrous cycle. When two normal cycles had been observed a smear was taken when the membrane was open. The day of maximum cornification before leucocytic influx was designated day 1 (Stockard & Papanicolaou 1917, 1919; Bland & Donoan 1970; Thorbert 1978).

Determination of conception day in pregnancy. 4-5 female were caged with one male. Day 1 of pregnancy was determined as the second day in the last period of open vaginal membrane (Kelly & Papanicolaou 1977; Phoenix 1970). Gestational length was controlled by palpation early in pregnancy.

Experiment 1: Resting and taken in the estrous cycle. At day 16-2, 3-5, 6-7, 8-11 and 1-15 1 experiment on pregnant animal were undertaken at day 6-8, 16-18, 28-3, 38-4, 48-5, 63-68 and 4-1 h post partum. In animal used for post partum experiment, labour was sometime induced with oxytocin (2 IFU/ml) occasionally repeated once (Phoenix 1970).

Dissection and macrodissection procedure. The animal was stunned and bled. In pregnant animal the fetuses, placenta and membrane were removed. The organs were then mounted according to Hammarström & Sjöstrand (1979). Horns from animal taken from experiment at pregnancy day 40 or later were mounted in organ bath of 100 ml volume.

Hormone treatment. Three weeks after castration treatment was either a triadial valeric (Progynon) 6 mg/kg or hydroxyprogesterone caproate (Proluton®) 150 mg/kg or both hormones every second day was started and continued for 10-14 days.

Experimental performance. Experiment were performed following the schedule given by Hammarström & Sjöstrand (1979).

After 60 min accommodation in ordinary Tyrode solution (36°C) changed every 70 min the sampling was started. Five sampling periods, lasting for 10 min in Tyrode solu-

tion where glucose was replaced by NaCl formed. The periods were interrupted by use of 70 min in ordinary Tyrode solution. First, the sampling period was used for determination of resting secretion level. The second was used for studying the effect of continuous stimulation or administration of drugs or both. In these cases there were two resting periods and one stimulation period.

Estimation of tissue carbohydrate concentration. As described in detail by Hammarström & Sjöstrand (1979). The secretory response was calculated as percent increase in carbohydrate secretion above secretion level using the mean of the resting levels as matching value. Concerning the large (from late pregnancy experiments) the following conditions were introduced. The samples were kept in thermostatic bath (40°C). The dry residue was double the amount of water and 14.4 M H_2SO_4 (4.5 and 9 ml respectively). After centrifugation 45 supernatant was removed and 0.15 ml 1% NaOH was added. The values of secretion level were for the dilution. Recovery of added glucose (10^{-4} $\times 10^{-4}$ M) yielded 67% (V.S.D. Recovery corrected for the dilution yielded 81% (V.S.D. $n=8$).

Determination of endometrial carbohydrate. After the experiment the endometrial layer was cut from the myometrium and a small piece was taken for analysis. Each piece was homogenized by pulv. quartz sand in a mixer containing 0.5 ml of 11 N. Rinsing was performed with 4 ml 14.4 M H_2SO_4 .

Table 2 Endometrial carbohydrate concentration and resting secretion level during pregnancy. Mean \pm S.D. number of experiments (data not shown)

Day of pregnancy	Endometrial carbohydrate concentration ($\mu\text{mol/g}$)	Resting secretion level ($\mu\text{mol}/10 \text{ min}$)
6-8	4 ± 11 (14)	0.71 ± 0.36 (1)
16-18	33 ± 11 (7)	1.6 ± 0.1 (1)
28-3	35 ± 1 (9)	0.71 ± 0.36 (1)
38-4	33 ± 4 (8)	0.60 ± 0.4 (1)
48-5	35 ± 15 (13)	0.95 ± 0.41 (1)
63-68	38 ± 16 (17)	1.40 ± 0.21 (1)
4 h p.p.	30 ± 12 (17)	0.50 ± 0.36 (1)

This value is higher ($P < 0.01$) than values at day 6-8 and 4 h p.p.

This value is higher ($P < 0.001-0.05$) than all values in the group.

The level is higher ($P < 0.001$) than the level of groups except the day 63-68 group.

The level is higher ($P < 0.01$) than the level of groups except for day 16-18 group and day 48-5 p.p.

This value is lower ($P < 0.01$) than that of day 48-5 group.

Table 1 Endometrial carbohydrate concentration and resting secretion level during the estrous cycle

There was no statistically significant difference between the groups. Mean \pm S.D. number of experiments within brackets

Day in estrous cycle	Endometrial carbohydrate concentration ($\mu\text{mol/g}$)	Resting secretion level ($\mu\text{mol}/10 \text{ min}$)
16–	50 ± 18 (18)	0.74 ± 0.4 (1)
3–5	43 ± 16 (5)	0.71 ± 0.5 (18)
6–7	49 ± 16 (14)	0.61 ± 0.36 (13)
8–11	51 ± 17 (13)	0.85 ± 0.7 (14)
1–15	43 ± 8 (1)	0.75 ± 0.36 (10)

animals were controlled daily concerning the status of the vaginal closure membrane. Only animals showing regular cycles were used.

Animals used for the study of hormone effects were oophorectomized under sodium pentobarbital anaesthesia (30–40 mg/kg) (when necessary supplemented with ether).

Determination of oestrus in the estrous cycle. When two normal cycles had been observed a smear was taken when the membrane was open. The day of maximum cornification before leucocytic influx was designated day 1 (Stoekard & Papanicolaou 1917, 1919; Bland & Donovan 1970; Thorbert 1978).

Determination of conception day in pregnancy. 4–5 females were caged with one male. Day 1 of pregnancy was determined as the second day in the late period of open vaginal membrane (Kelly & Papanicolaou 1977; Phoenix 1970). Gestational length was controlled by palpation early in pregnancy.

Experiment on resting secretion level in the estrous cycle. At day 16–3–5–6–7–8–11 and 1–15. Experiment on pregnant animals were undertaken at day 6–8–16–18–8–3–38–4–48–5–63–68 and 4–1 h post partum. In animals used for post partum experiment labour was sometimes induced with oxytocin (1 P i.m. occasionally repeated once) (Phoenix 1970).

Dissection and mounting procedure. The animals were stunned and bled. In pregnant animals the fetuses, placentae and membranes were removed. The organs were then mounted according to Hammarström & Sjöstrand (1979). Horns from animal taken from experiments at pregnancy day 40 or later were mounted in an organ bath of 100 ml vol. me.

Hormone treatment. Three weeks after castration treatment with either estradiol valerate (Progynon) 6 mg/kg or hydroxyprogesterone caproate (Prohuton) 150 mg/kg or both hormone every second day was started and continued for 10–14 days.

Experimental performance. Experiments were performed following the schedule given by Hammarström & Sjöstrand (1979).

After 80 min accommodation in ordinary Tyrode solution (36°C) changed every 70 ml the sampling was started. Five sampling periods lasting for 10 min in Tyrode solu-

tion where glucose was replaced by NaHCO_3 formed. The periods were interrupted by recovery of 70 min in ordinary Tyrode solution. First and last sampling period was used for determination of resting secretion level. The second half was used for studying the effect of continuous stimulation or administration of drugs in later experiments only three sampling periods were used. In these cases there were two resting periods or one stimulation period.

Estimation of at rise carbohydrate secretion. Formed as described in detail by Hammarström (1979). The secretory response was calculated as percent increase in carbohydrate secretion above secretion level using the mean of the resting secretion as matching value. Concerning the steps (from late pregnancy experiments) the following modifications were introduced. The samples were kept in a thermostatic bath (40°C). The dry residue was double the amount of water and (4.5 M H_2SO_4) and 9 ml respectively. After centrifugation (15 s) supernatant was removed and 0.15 ml H_2SO_4 was added. The values of secretion level were for the dilution. Recovery of added glucose was $10 \times 10^{-4} \times 10^{-4}$ yielded $67 \pm 6\%$ (V.S.B.). Recovery corrected for the dilution yielded $88 \pm 5\%$ (S.D. $n=8$).

Determination of endometrial carbohydrate. After the experiment the endometrial layer was removed from the myometrium and a small piece was taken for analysis. Each piece was homogenized by grinding in a mortar containing 0.5 ml of 14 M HCl. Grinding was performed with 4 and 14 M HCl, 50°C.

Table 2 Endometrial carbohydrate concentration and resting secretion level during pregnancy. Mean \pm S.D. number of experiments within brackets

Days of pregnancy	Endometrial carbohydrate concentration ($\mu\text{mol/g}$)	Resting secretion level ($\mu\text{mol}/10 \text{ min}$)
6–8	42 ± 11 (14)	0.71 ± 0.6 (6)
16–18	53 ± 11 (7)	1.6 ± 0.1 (6)
28–32	35 ± 1 (9)	0.73 ± 0.6 (6)
38–4	33 ± 4 (8)	0.60 ± 0.2 (6)
48–52	35 ± 15 (13)	0.93 ± 0.4 (6)
63–68	38 ± 16 (17)	1.40 ± 0.3 (14)
4 h p.p.	30 ± 12 (17)	0.50 ± 0.2 (14)

This value is higher ($P < 0.01$) than values at 16–18 and 4 h p.p.

This value is higher ($P < 0.001$ – 0.05) than all values in the group.

The level is higher ($P < 0.001$) than the level of all groups except the day 63–68 group.

The level is higher ($P < 0.01$) than the level of all groups except for day 16–18 group and day 48–52 group.

This value is lower ($P < 0.01$) than that of all groups.

weight endometrial carbohydrate concentration and resting secretion level of uteri from guinea-pigs given and/or progesterone

III. number of experiments within brackets. Concerning uterine wet weight, there were statistically significant differences between the groups. No such differences were seen with respect to the endometrial carbohydrate concentration. Resting secretion level of combined treatment group was lower ($P < 0.01$) than that of the combined groups

	Uterine wet weight (g)	Endometrial carbohydrate concentration ($\mu\text{mol/g}$)	Resting secretion level ($\mu\text{mol/10 min}$)
and progesterone treatment	0.73 ± 0.15 (23)	44 ± 16 (19)	0.47 ± 0.17 (7)
treatment	0.91 ± 0.40 (30)	39 ± 14 (11)	0.77 ± 0.35 (20)
combined treatment	0.86 ± 0.14 (14)	42 ± 26 (10)	0.60 ± 0.23 (12)
combined treatment versus estrogen treatment	$P < 0.05$	n.s.	$(P < 0.01)$
combined treatment versus progesterone treatment	$P < 0.001$	n.s.	n.s.
combined treatment versus progesterone treatment	$P < 0.001$	n.s.	n.s.

After shaking the homogenate was centrifuged at 4500 g for 10 min. The supernatant was removed and to it 8.5 ml of 1 M acetic acid was added. The sample was then analysed by the carbonyl method (Schmidt & Matthies 1963).

After shaking the homogenate was centrifuged at 4500 g for 10 min. The supernatant was removed and to it 8.5 ml of 1 M acetic acid was added. The sample was then analysed by the carbonyl method (Schmidt & Matthies 1963).

stimulation was applied at standard rate of 0.5 mA, 30 V which had been found to give optimal results (vide Hammarström & Sjöström 1979). At every day 16–18, 28–32, 38–42, the uterine fluid was aspirated from the placental and fetuses were expelled.

made it impossible to obtain an adequate spread of the field stimulation experiments are outlined on day

Apart from the hormones obtained from Schering (carboxyphenylolone chloride (Carbachol) (Sigma), luteal hydrobromide (Sigma), phenolamine de (Ciba), methoxamine hydrochloride (BDH), and cml (Ferrostat) were used.

the Students' *t*-test or when necessary the approximate *t*-test (vide Renwick & Schork 1968) as used for statistical comparison between the

RESULTS AND COMMENTS

uterine carbohydrate concentration (Tables 1 and 2 and 3)

throughout the estrous cycle and after hormone treatment little variation in mucosal carbohydrate concentration was seen. The similarity in mucosal carbohydrate concentration is probably explained by the fact that female sex hormones alone or in combination change the relative distribution and

type of polysaccharides in epithelial glandular or stromal tissues rather than affect total carbohydrate concentration as such (cf Schmidt & Matthies 1963).

Secondary during pregnancy mucosal carbohydrate concentration was rather uniform. Maximum concentration was noticed in early pregnancy (day 16–18).

Resting carbohydrate secretion level (Tables 1, 2 and 3)

During the estrous cycle no statistically significant variation in resting secretion level was noted. In pregnancy maximum secretion level was noted at day 16–18 when mucosal carbohydrate concentration was largest (see above). A second maximum was noted at day 63–68 of pregnancy. No significant difference in resting secretion level between the groups receiving single hormone treatment was noted. However the group receiving combined treatment had lower resting secretion level.

Field stimulation response (Figs 1 and 2)

Estrous cycle (Fig 1A.) A secretory response to field stimulation was elicited at the days around ovulation i.e. estrus (day 16–2) (+20%). On day 3–5 (metestrus) field stimulation caused an inhibition of carbohydrate secretion (–15%). The discrepancy in effect on carbohydrate secretion caused by field stimulation and carbachol (see below) respectively together with previous results that possibly

FIELD STIMULATION OF NERVES

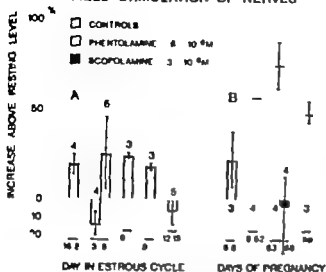


Fig. 1. Secretory responses (carbohydrate release) of guinea-pig uteri to transmural stimulation during estrous cycle (A) and during pregnancy (B). Uteri were stimulated continuously with 0.5 Hz 0.5 ms 30 V during 10 min periods. Responses in this and following figures are expressed as per cent of resting secretion level, i.e. carbohydrate release during control periods. For further explanation see Material and Method. (A) Responses are seen at all intervals except late diestrus (day 1-15). Note the reversed answer after phentolamine administration at day 3-5. (B) A response is seen at time of implantation (day 6-8). A greater response is seen in late pregnancy. The response period is post partum. Scopolamine abolishes the response at day 63-68 (mean and range number of experiment indicated by figures at column).

might suggest an inhibitory adrenergic influence on the uterine carbohydrate secretion (vide Figs. 1 and 3 in Hammarström & Sjöstrand 1979) prompted a test with the α -blocker phentolamine. This drug unmasked a secretory response of about $+5\%$, i.e. of the same magnitude as that elicited by carbachol. Secretory responses were obtained from day 6 until day 11 (early diestrus) of the estrous cycle ($+20\%$) without treatment with phentolamine. In late diestrus (day 1-15) (i.e. when serum levels of progesterone are low (Eder, Resko & Goy 1968; Croix & Franchimont 1975)) and when epithelium is atrophic (low and cuboidal (Stockard & Papanicolaou 1917; Burgos & Wislocki 1956, 1958)) no secretory response could be noted.

Pregnancy (Fig. 1B). At time of implantation (day 6-8) a secretory response was elicited ($+20\%$). From time of placentation (at about day 10) until day 40 field stimulation was impossible to accomplish (see Material and Methods). In late pregnancy the secretory responses were of greater mag-

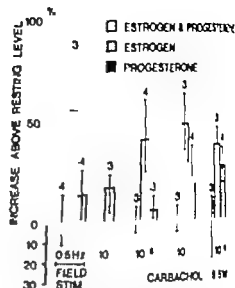


Fig. 2. Effects of field stimulus (0.5 Hz) and carbachol on carbohydrate secretion from guinea-pig uteri treated with excess of either progesterone. Responses are not seen in the progesterone-treated group but in the estrogen-treated group. Responses are seen in the group receiving both (cf. responses of pregnant uteri Fig. 1 and 4).

nitude and reached $+57\%$ at day 40 and day 65. 4-12 h post partum a response could be noted ($+47\%$) (i.e. when the parturient is anesthetized and resuscitation can take place (Franchimont & Sjöstrand 1979)). Scopolamine abolished the secretory response at day 63-68. This is a further confirmation of the cholinergic, muscarinic nature of the response (cf. Hammarström & Sjöstrand 1979).

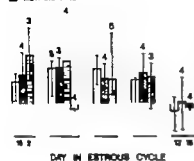
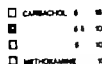
Hormone treatment (Fig. 2). Field stimulation produced an increase in carbohydrate release above resting level in uteri from animals treated with estrogen only ($+14\%$). The increase was, however, slight compared to that of uteri from animals receiving estrogen as well as progesterone ($+49\%$).

No clearcut response to field stimulation was obtained in uteri from animals receiving progesterone only.

Effect of carbachol (Fig. 3 and 4)

Estrous cycle (Fig. 3). Carbachol (5.5×10^{-5} M) rendered a secretory response was roughly parallel to that elicited by field stimulation ($+20\%$). Consequently no secretory responses were seen in late diestrus (cf. above).

Pregnancy (Fig. 4). At day 6-8 of pregnancy a secretory response ($+20\%$) could be noted. At



Carbohydrate release from guinea-pig uteri in response to carbachol during estrous cycle. As was the case with field stimulation (Fig. 1) secretory responses are present throughout the estrous cycle except for late diestrus. No response was noted after methoramine at day 3-5.

No certain response was detected. This might be due to the fact that at this time a rather small area of endometrium persists after the placental membranes have been removed. Furthermore, the "resting secretion level" might conceal a secretory response (cf. below).

In mid-pregnancy (day 28-32) a response of about 50% was noted. In late pregnancy (day 48-68) an even greater secretory response was noted (about

+60%). The secretory response persisted after delivery (+45%).

The secretory responses to carbachol (5.5×10^{-4} – 5.5×10^{-6} M) reached approximately the same level as those corresponding to field stimulation.

Hormone treatment (Fig. 1). In the combined treatment group as well as in the estrogen treated group carbachol raised the carbohydrate secretion to the same extent as field stimulation. Compared to the combined treatment group the response was shifted to the right by more than a power ten in the estrogen treated group.

Even very high concentrations of carbachol (5.5×10^{-4} M) were without overt effect on carbohydrate secretion from uteri of animals treated with progesterone only.

Methoramine at a concentration of 4×10^{-4} M was without overt effect on the carbohydrate secretion at day 3-5 in estrous cycle (Fig. 1).

DISCUSSION AND CONCLUSIONS

General criticism of the method has been discussed earlier (Hammarstrom & Sjöstrand 1979). Naturally, concerning this study it should be recalled that as the endometrium definitely exhibits great functional and morphological changes according to its hormonal state, a marker of secretion not subjected

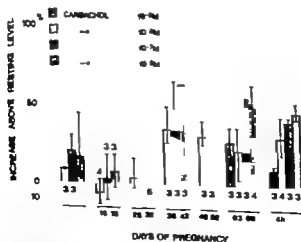


Fig. 4. Carbohydrate release from guinea-pig uteri in response to carbachol during pregnancy. Secretory responses are roughly parallel to those obtained after field stimulation (cf. Fig. 1). However, this figure also includes experiments at mid pregnancy (day 23-32 and 34-42).

to hormonal regulation is hardly possible to find. Here it should be mentioned that the measured secretory responses have a drawback constituted by the uncertainty of what "resting secretion level" actually represents, e.g. at days 16–18 of pregnancy the high resting secretion level might disguise a secretory response.

The question of what resting secretion level represents has to be touched. The small variation in carbohydrate resting secretion level of uteri from animals treated with excess of estrogen or progesterone is remarkable. Possibly quite a proportion of the resting secretion represents a constant carbohydrate leakage from all types of uterine tissue. Concerning the lack of differences in resting secretion level between most groups it should also be recalled that changes in secretory products, e.g. from glycogen to mucopolysaccharides may occur. Such changes occur regularly in the late secretory phase in the human endometrium (Cramer & Klöss 1955, Runge, Ebner & Lindenschmidt 1956, Schmidt-Matthiesen 1963). Concerning the peak in resting secretion level at pregnancy day 16–18 the proportionally greater endometrial area that is occupied by placental sites should be considered. The ulcerated sites might render a greater leakage of carbohydrate. The second maximum observed at day 63–65 possibly merely reflects the increase in size of all uterine tissue.

One general conclusion seems to be justified by the results in this paper, i.e. the responses studied are dependent on estrogen as well as progesterone. Thus uteri of animals receiving both hormones showed larger responses and were also more sensitive to carbachol than uteri of animals receiving estrogen only. It is well documented that both for development and maintenance of the secretory phase of the endometrium both estrogen and progesterone are necessary. Yet endometrium of the guinea pig receiving excess estrogen only responded to nerve stimulation as well as to direct muscarinic stimulation. This would be in line with the electron microscopical findings of e.g. Cramer & Klöss (1955), Wessel (1960), Wetzstein & Wagner (1960), Nilsson (1967), Wynn & Harris (1967) showing similar but minor discharge of secretory products during the proliferative phase to that occurring in the secretory phase. Neither nerve stimulation nor muscarinic stimulation of the endometrium of animals treated with excess progesterone only produced any definite response in the studied

parameters. Whether this was solely due to lack of secretory cells to respond to stimuli or alterations in the neuro-effector response is left veiled.

The secretory responses studied roughly parallel the serum progesterone level during the oestrous cycle and during pregnancy (Heap & Deane 1966, Feder et al. 1968, Challinor, Heap & Illingworth-Cox 1974, Croix & Franchimont 1975). Serum samples of the guinea pig are difficult to analyze during oestrous cycle because they are very low and are marked by individual variations throughout the cycle (Challinor et al. 1971, Croix & Franchimont 1975). However, towards end of pregnancy low serum levels are seen (Challinor et al. 1971) and during the oestrous cycle a secretory response to nerve stimulation or carbachol could be seen except for the late diestrous phase when secretory progesterone is low. A secretory response to stimulation or carbachol was present during pregnancy as well as post partum. The maximal response in late pregnancy was similar to that of uteri treated with excess of estrogen and progesterone. This makes sense since towards end of pregnancy both estrogen and progesterone are at maximum levels (Heap & Deane 1966, O'Connor et al. 1971).

The results also suggest that the cholinergic secretory innervation persists during gestation in respect to the fact that the myometrial secretory innervation disappears during pregnancy (O'Connor 1967, Owman et al. 1974, Thorbert 1978) and that uteri in late pregnancy and post partum responded to field stimulation points to fundamental differences between adrenergic, myometrial and endometrial cholinergic nerves.

Evidence of an adrenergic, possibly neuroendocrine inhibition of the cholinergic secretory response to intramural stimulation was obtained at different stages of the oestrous cycle. Whether this inhibition is pre- or postjunctional or both is at present difficult to say. Further solution of this question would require more sophisticated methods. Anyhow adrenergic nerves to the basal endometrium of several species have been reported (vide e.g. Adham & Cullen 1969, Dallenbach & Vonderlin 1973, Thorbert 1977). The adrenergic innervation is dependent on female sex hormones (Falck et al. 1974, Owman et al. 1974, Thorbert, Alm & Rosengren 1979) particularly the relative level of estrogen versus progesterone which might be the reason for the adre-

ly response revealed only at day 3-5 in estrus (cf. Thorbert 1978; Thorbert et al. 1978).

It should be emphasized that secretory responses could be elicited at all crucial events of reproductive cycle of the female guinea-pig, i.e. estrus, days of implantation, period of fetal and the end of pregnancy, well as postpartum when repeated fertilization naturally occurs. In conclusion it is challenging and urges to studies concerning the significance of the cholinergic secretory nerves.

I am grateful to Mr Jan Olof Hedborn for excellent technical assistance and to Mrs Anneli Rönkä for preparing the pictures and to Miss Eva Svermova for preparing the manuscript.

This work is supported by grants from Svenska Vetenskapsrådet and Medicinska Forskningsrådet.

REFERENCES

1. N. & SCHENK E. A. 1969 Anatomical studies of the rat vagina, cervix and uterus and its function. *Am J Obstet Gynecol* 104, 308-316.
2. R. F. & DONOVAN B. T. 1970 Oestrogen and progesterone and the function of the corpus luteum in the guinea pig. *J Endocrinol* 47, 223-230.
3. M. H. & WISLOCKI G. B. 1946 The cyclical changes in the guinea-pig uterus, cervix, vagina and skin investigated by histological and histochemical means. *Endocrinology* 49, 93-118.
4. M. H. & WISLOCKI G. B. 1948 The cyclical changes in the mucosa of the guinea pig uterus, cervix and vagina and in the vaginal skin, investigated by histochemical means. *Endocrinology* 63, 106-121.
5. J. R. O. HEAP R. B. & ILLINOWORTH J. 1971 Concentrations of oestrogen and progesterone in the plasma of nonpregnant, pregnant and lactating guinea pigs. *J Endocrinol* 51, 133-145.
6. E. H. & KLOSS O. 1955 Verbesserung der Histochemie am Endometrium durch Anwendung histochemische Darstellung der Geschlechtsorgane. *Arch Gynäkol* 185, 778-798.
7. D. & FRANCHIMONT P. 1975 Changes in the levels of the gonadotropins, progesterone and oestrogen during the estrous cycle of the guinea-pig. *Endocrinology* 79, 1-11.
8. ENBACH-HELLWEG G. 1969 *Endometrium*. Verlag Berlin.
9. ENBACH F. D. & VONDERLIN D. 1973 The physiology of the human endometrium. *Arch Gynäkol* 215, 372-378.
10. C. B. GÄRDMARK, S. NYBELL, G. OWMAN, ROSENQREN E. & SJÖBERG, N.-O. 1974 The influence of the context of neuroendocrine system on the guinea pig and rat uterus. *Endocrinology* 114, 175-179.
11. FEDER, H. H. RESKO J. A. & GOY R. W. 1968. Progesterone concentrations in the arterial plasma of guinea-pigs during the estrous cycle. *J Endocrinol* 40, 503-513.
12. HAMMARSTRÖM M. & SJÖSTRAND N. O. 1975 Evidence for cholinergic secretory innervation of the guinea-pig endometrium. *Acta Physiol Scand* 106, 11-15.
13. HEAP R. B. & DEANESLY R. 1966 Progesterone in systemic blood and placenta of intact and ovariectomized pregnant guinea-pigs. *J Endocrinol* 34, 417-423.
14. HITSCHMANN F. & ADLER L. 1908. Der Bau der Uterusmuskulatur des geschlechtstüchtigen Weibchens mit besonderer Berücksichtigung der Menstruation. *Monatsschrift Geburtshilfe Gynäkol* 27, 1-82.
15. KELLY H. L. & PAPANICOLAOU G. N. 1917 The mechanism of the periodical opening and closing of the vaginal orifice in the guinea-pig. *Am J Anat* 140, 387-411.
16. LAWN A. M. 1973 The ultrastructure of the endometrium during the estrous cycle. In: *Advances in reproductive physiology* vol. 6 (ed. M. V. H. Bishop) pp. 61-95. Academic Press, London.
17. MARSHALL J. B. 1970 Adrenergic innervation of the female reproductive tract. *Anatomy, physiology and pharmacology*. *Rev Physiol Biochem Pharmacol* 6, 6-67.
18. NILSSON O. 1966 Correlation of structure to function of the terminal cell surface in the uterine epithelium of mouse and man. *Z. Zellforsch Mikrosk Anat* 56, 803-808.
19. MOYES R. W. HERTIG A. T. & ROCK J. 1950. Dating the endometrial biopsy. *Fertil Steril* 1, 3-25.
20. OWMAN C. H. SJÖBERG, N.-O. & SJÖSTRAND N. O. 1974 Short adrenergic neurons, peripheral neuroendocrine mechanism for amino fluorescence histochemistry (ed. M. Fajers & C. Tanaka), pp. 47-66. Igaku Shoin Ltd, Tokyo.
21. PHOENIX C. H. 1970 Guinea pigs. In: *Reproduction and breeding techniques for laboratory animals* (ed. E. S. E. Hafez), ch. 14, pp. 244-257. Lea & Febiger, Philadelphia.
22. REMINGTON R. B. & SCHORK M. A. 1970 Statistics with applications to the biological and health sciences. Prentice-Hall, Englewood Cliffs, N. J.
23. RUNGE, H. EBNER, H. & LINDENSCHEIDT W. 1974 Vorgehens der kombinieren Alkalischen Periodik-Schicht Reaktion für die gynäkologische Histo-pathologie. *Dtsch Med Wochenschr* 38, 15, 5-1529.
24. SCHMIDT MATTHIEN H. 1963 Histochemie I. Das normale menschliche Endometrium (ed. H. Schmidt-Matthiesen), pp. 149-224. H. Thieme Verlag, Stuttgart.
25. SJÖBERG N.-O. 1967 The adrenergic innervation of the female reproductive tract: distribution and functional changes. *Acta Physiol Scand* 72, Suppl. 305, 1-32.
26. STOCKARD C. R. & PAPANICOLAOU G. N. 1917 The existence of a typical estrous cycle in the guinea-pig — with a study of its histological and physiological changes. *Am J Anat* 22, 223-283.

- STOCKARD C R. & PAPANICOLAOU G N 1919 The vaginal closure membrane and the vaginal plug in the guinea pig with further considerations of the oestrous rhythm. *Biol Bull Mar Biol Lab Woods Hole* 37 22-45
- THEMANN H & SCHÜNKE W 1963 Die Feinstruktur der Drüsenepithelien des menschlichen Endometrium Elektronoptische Morphologie. In *Das normale menschliche Endometrium* (ed H Schmidt Matthies) pp 111-148. Thieme Verlag Stuttgart
- THORBERT G 1978 Regional changes in structure and function of adrenergic nerves in guinea-pig uterus during pregnancy. *Acta Obstet Gynecol Scand Suppl* 79 1-3
- THORBERT G, ALM P & ROSENGREN E 1978 Cyclic and steroid-induced changes in adrenergic neurotransmitter level of guinea-pig uterus. *Acta Obstet Gynecol Scand* 57 45-48
- THORBERT G, ALM P, ÖWMAN C & STRÖM N-O 1977 Regional distribution of adrenergic nerves in guinea-pig uterus. *Am J Physiol* 233 C5-C9
- WESSEL W 1960 Das elektromikroskopische menschlische endometrialer Drüsen während menstruellen Zyklus. *Z Zellforsch Mikrosk* 51 631-657
- WETZSTEIN R. & WAGNER H 1960 Mikroskopische Untersuchungen am menschlichen Endometrium. *Anat Anz* 108 36-374
- WYNN R. M. & HARRIS J. A. 1967 Uterine cyclic changes in the human endometrium in the preovulatory phase. *Fertil Steril* 18 632-648

Extracellular calcium as a determinant of action potential duration in rabbit myocardium

Ulf JOHANSSON and Björn WOHLFART

Department of Pharmacology, University of Iceland, Reykjavík, Iceland and Department of Pharmacology, University of Lund, Lund, Sweden

Johansson, U. & Wohlfart, B. Cellular calcium as a determinant of action potential duration in rabbit myocardium. *Acta Physiol Scand* 1980, 110: 241-247. Received 28 Nov 1979. ISSN 0001-6772. Department of Pharmacology, University of Iceland and Department of Pharmacology, University of Lund, Sweden.

It has been suggested that the amount of calcium in the myocardial cell determines force production as well as the duration of the action potential. We have examined two factors proposed to determine the action potential duration: (1) the inotropic state of the muscle and (2) the preceding stimulation interval. The experiments were performed on isolated papillary muscles from rabbits. The preparations were stimulated to contract isometrically at a temperature of 37°C and intracellular recordings of action potential were made. An increased inotropic state produced either by raised external calcium concentration or by increased stimulation frequency was associated with shortened action potentials, confirming factor (1) above. The dependence of the action potential duration on the preceding stimulation interval (factor (2) above) disappeared over a wide range of interval when the preparation was exposed to 4 µM D600. The action potential duration was, however, still dependent on the preceding stimulation interval when the extracellular calcium concentration had been lowered from 2.0 mM to 1.0 and 0.5 mM respectively. The results are discussed in terms of a model which postulates an inverse relationship between the amount of calcium in a membrane compartment and the action potential duration.

Key words: Rabbit papillary isometric force, action potentials, low calcium D600.

generally held that the intracellular concentration of free calcium determines the contractile force of the myocardial cell (Ebashi & Endo 1968; Fozzard 1977). It has furthermore been suggested that the extracellular calcium concentration controls the duration of the action potential in cardiac muscle (Isenberg 1975; Kass & Tsien 1976; Spillmann, Fry & McGowan 1976; Boyett 1977). Such that increased concentration of calcium in the cell leads to abbreviation of the action potential in accordance with this an inverse relationship between contractile strength and action potential duration has often been observed (Trautwein & Boyett 1954; Edmonds, Greenspan & Fisch 1968; & Suckel 1968; Edman & Johansson 1976; Johansson 1978). It was proposed in a previous study (Johansson 1979) that the action potential duration is mostly determined by the inotropic state of the muscle but also depends on the length of the preceding

stimulation interval. The influence of these two factors on the action potential was explained by assuming that a membrane compartment is gradually filled with calcium during the time interval between stimuli and that the degree of filling inversely determines the action potential duration. The present study has been aimed at further testing this idea. To this end we have paced isolated papillary muscles with different pacing protocols in order to produce changes of the action potential duration. Under these conditions we have also varied the calcium concentration in the bathing solution and compared the effects with those of the calcium antagonist D600 which is known to block the calcium influx through the cell membrane.

METHODS

Preparation and mounting. Papillary muscles were dissected from the right ventricles of rabbits (weight about 1 kg).

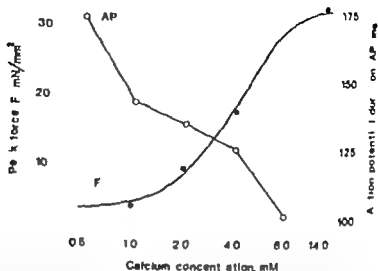


Fig. 1 Peak isometric force (F) and action potential duration (AP) of an isolated papillary muscle (paced at 1 Hz) in relation to the calcium concentration in the perfusate. Note the inverse relationship between F and AP as the calcium concentration is changed.

The techniques used for dissection and mounting of the muscles have been described in detail (Edman & Jóhannsson 1976). The heart was removed and split open in a warm oxygenated solution. Papillary muscles (largest diameter 0.3–0.7 mm, length 3–5 mm) were excised and loops of platinum wire were tied to the tendon and to a piece of the ventricular wall at the base of the muscle. The muscle was mounted horizontally in a thermostatically controlled bath (4 ml) between two hooks, one of which was fitted to the lever of a force transducer. The position of the other hook was controlled by means of a micrometer screw for fine adjustment of the muscle length. The length was set to 95% of the length at which maximal isometric force was obtained.

Force transducer. Force was measured by means of a semiconductor strain-gauge transducer. The free resonant frequency of the transducer was approximately 700 Hz. A linear response was obtained for forces up to at least 50 mN. The compliance of the transducer was less than 2 $\mu\text{m}/\text{mN}$. The transducer signal was recorded on a polygraph ink writer and displayed on a storage oscilloscope (Tektronix 5103N).

Recording of membrane potential. Membrane potentials were recorded by means of a conventional microelectrode technique. The glass capillaries were filled with 3 M KCl and had a resistance of 20–30 M Ω . The microelectrode and an Ag/AgCl reference electrode were connected differentially to an amplifier with high input impedance and capacitance neutralization. The signals were displayed on the storage oscilloscope and photographed on 35 mm film. The film records were analysed at 10 times magnification. The action potential duration was measured at a voltage level of -40 mV.

Stimulation. The muscle was stimulated by passing current through a pointed electrode (platinum wire) placed over the base of the muscle. The current intensity exceeded the threshold value by 30–50%. Current was de-

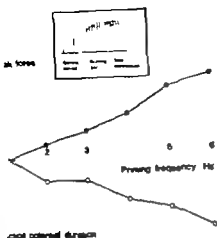
livered from an electrically isolated power amplifier, which was operated by a Grass 8 multistage. The amplifier was gated by a programmable control unit which was able to pre-set different stimulation sequences $\pm 5\%$.

Perfusion. The muscle was continuously perfused (ml/min) with a bath solution of the following composition (mM): NaCl 100.0, KCl 4.0, NaHCO_3 20.0, NaH_2PO_4 1.5, MgSO_4 1.5, CaCl_2 0.0, Na-acetate 20.0 and glucose 10.0. Insulin 2 IU per liter was added. The solution was equilibrated with a gas mixture containing 95% O_2 and 5% CO_2 , yielding a pH of 7.4. All chemicals used were analytical grade. D600 was kindly supplied by Karl Ludwigshafen, Germany. The water used for washing glassware and for preparation of solutions was distilled. The bath temperature was 36–37°C. In any given experiment temperature varied by less than $\pm 0.2^\circ\text{C}$.

RESULTS

1 Action potentials in relation to the isotropic state and the preceding stimulation interval

It was reported in a previous study (Wohlfart 1975) that the action potential duration was inversely related to (1) the isotropic state of the muscle and (2) the length of the preceding excitation interval. These relations have been further examined in the present study. In the experiment demonstrated in Fig. 1, the isotropic state of the muscle was changed by varying the calcium concentration in the bathing fluid. The muscle was continuously paced at 1.0 Hz and was allowed to equilibrate for at least 15 min



peak force and action potential duration of the test contraction, marked by an arrow in the inset, related to action frequency during the preceding priming (inset) (interval between stimuli in the control is before the test contraction was 1.0 s. Note the relationship between contractile force and action duration.

edge of the calcium concentration. It can be seen in Fig. 1 that peak isometric force (F) varied with the calcium concentration whereas action potential duration (AP), measured at about $\frac{1}{4}$ (see Methods) decreased. Thus, AP and F varied inversely as the external calcium concentration was altered.

This inverse relationship was observed during post-stimulation potentiation. The stimulation sequence used is illustrated by the inset of Fig. 2. The muscle was stimulated at a frequency of 1.0 Hz. This was followed by a 2 s period during which a train of 16 stimuli of $\frac{1}{4}$ frequency was given. A test stimulus was given 1.0 s after the last stimulus in the priming period.

It can be seen in Fig. 2 that with increasing frequency during the priming period peak isometric force (F) of the test contraction increased whereas action potential duration (AP) at the same time decreased.

Fig. 3A demonstrates how the action potential duration is affected by changes of the preceding stimulation interval. The following stimulation sequence (see inset of the figure) was used. The muscle was stimulated at a regular frequency of 1 Hz. Three control contractions are referred to as 1, 2 and 3. At a given moment the sequence was interrupted and two test pulses, designated 1 and 2, were applied. The first test interval,

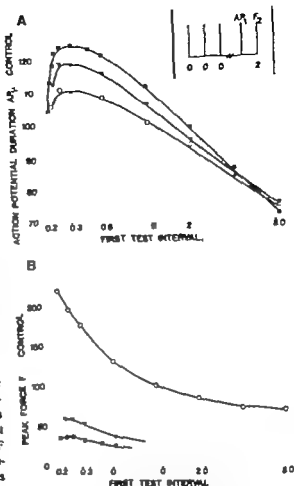


Fig. 3 (A) The action potential duration of the first test contraction (AP_1) in relation to the preceding stimulation interval. For the pacing sequence see inset and Results, section 1. The external calcium concentration was 2.0 mM (\circ), 1.0 mM (\blacktriangle) and 0.5 mM (\blacksquare). (B) Peak force of the second test contraction (F_2) after the varied stimulation interval. Pacing sequence and symbols as in panel A.

the time from the last stimulus of the control period to the occurrence of test pulse 1 was varied (between 0.2 and 8.0 s). The interval between the first and the second test pulses was fixed at 1.0 s. The effects of this stimulation sequence on contractile force and action potential configuration were described and analysed in the previous study (Wohlhart 1979). Some effects will be commented on below. It is demonstrated by the open symbols in Fig. 3A that the action potential duration of the first test contraction (AP_1) decreases as the test interval is prolonged from about 0.3 s to 8.0 s. For shorter intervals than 0.3 s, AP is reduced

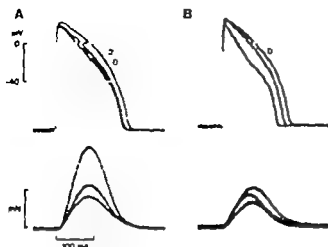


Fig. 4. Oscilloscope records of action potentials and isometric myograms from the same experiment as that demonstrated in Fig. 3. The external calcium concentration was 0 mM. The recordings from the two test contractions (designated 1 and 2) are superimposed on a control record (designated 0). The first test interval was 0.3 s in panel A and 8.0 s in panel B.

The greatest changes in force production occur in the second test contraction with this stimulation sequence. Fig. 3B (open symbols) shows that peak force of the second test contraction (F_2) is similarly related to the interval as AP₁ and thus reduced for the longer intervals.

Fig. 4 exemplifies original records of action potentials and isometric myograms of the two test contractions. For comparison the records are superimposed on the control traces. It can be seen that peak force of the first test contraction (F_1) is reduced both after a shortened (Fig. 4A) and a lengthened (Fig. 4B) test interval. The inverse relationship between peak isometric force and action potential duration is thus not seen in contraction 1 where the nearest preceding excitation interval is varied.

2. The effects of low calcium concentration on the relation between action potential and the preceding interval

The curve relating the action potential duration of the first test contraction (AP₁) to the preceding interval (Fig. 3A) was shifted towards longer action potentials when the external calcium concentration was reduced from 2.0 mM to 1.0 mM and 0.5 mM. This is in accordance with the results shown in Fig. 1. However, after the very short (about 0.2 s) and after the very long (about 8.0 s) intervals there was no significant difference be-

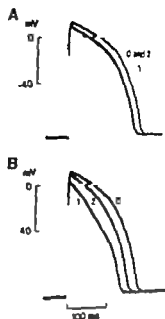


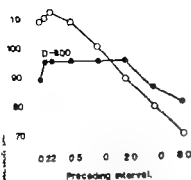
Fig. 5. Oscilloscope records of action potentials from the same experiment as that in Fig. 3 and 4. The external calcium concentration was 0.5 mM. Numbers of same pacing sequence as before (Fig. 3A, and 3B). The first test interval was 0.3 s in panel A and 8.0 s in panel B. It can be seen that the changes in action potential duration are not those seen in Fig. 4.

tween the curves in the different calcium concentrations. The well known negative inotropic effect of lowering the external calcium concentration, seen in Fig. 3B, where peak force of the second contraction (F_2) has been related to the level of AP₁, is not seen in Fig. 5B.

Fig. 5 shows original records of action potentials in an external calcium concentration of 0.5 mM. It can be seen that the effects of varying the test interval are similar to those seen in a calcium concentration of 2.0 mM (cf. Fig. 4). Thus the action potential of the first test contraction was very short in duration after a short interval (Fig. 5A) and prolonged after a long interval (Fig. 5B). When the calcium concentration was reduced below 0.5 mM the force production was very much reduced and the effect was not completely reversible. These experiments were therefore discarded.

3. The effects of D600 on the action potential duration

The agent D600 is known to block the slow inward current (Kohlhardt et al. 1977; Kass & Tsien, Nawrath et al. 1977) without any substantial effect on the duration of the action potential. A concentration of 4 μ M was used in the present experiments. This concentration reduced



Action potential duration of the first test contraction of the preceding stimulation interval (○) and after (●) the addition of $4 \mu\text{M}$ D600 to the muscle. Note that the action potential duration is the same over a wide range of test interval when the muscle is exposed to D600.

nic force to about 10% of the control value 20 min of exposure (stimulation rate 1.5 Hz). It is found that D600 made the action potential duration independent of the preceding stimulation interval over a wide range. It can be seen in Fig. 6 the action potential of the first test contraction (change in duration as the interval was varied from 0.25 s and 2.0 s. The same phenomenon is observed in all other preparations. Fig. 7 demonstrates action potential recordings of the two test contractions in a preparation exposed to D600. The traces in panel A are identical with the control (cf. Fig. 4B and 5B). The action potential recorded after a long test interval (8.0 s; panel B) is slightly shorter than the control (cf. Fig. 4B and 5B).

DISCUSSION

Action potential of mammalian myocardium is initiated by transmembrane currents carried by Na^+ , calcium, potassium and probably also chloride ions (Trautwein 1973; Noble 1975). A fast inward sodium current accounts for the initial rapid depolarization of the cell (Beeler & Reuter 1970; Trautwein 1972). A slow inward current is taken up during the plateau phase of the action potential (Beeler & Reuter 1970b; Trautwein 1972a and b). Sodium ions may participate in this current. A voltage and time-independent potassium current contributes to the repolarization of the cell. A time-independent potassium current has also been described that

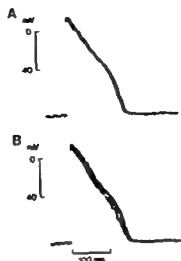


Fig. 7. Oscilloscope records of action potentials during the first (AP_1) and second (AP_2) test contraction superimposed on control record. The muscle was exposed to $4 \mu\text{M}$ D600. The pacing sequence was the same as before (Fig. 3A, inset). (A) Test interval: 0.3 s. Note that all three action potentials (control, AP_1 and AP_2) are practically identical. (B) Test interval: 8.0 s. AP_1 is slightly shortened whereas control and AP_2 are identical.

provides a constant background against which other ions move (McDonald & Trautwein 1978a and b). The action potential duration is believed to be determined by the decline of the slow inward current along with changes in potassium currents (Noble 1975; Bassingthwaite, Fry & McGuigan 1976).

It was reported in a previous study (Wohlfart 1979) that the action potential duration was inversely related to (1) the mototropic state of the muscle and (2) the length of the preceding stimulation interval. These relations have now been further studied and will be discussed in relation to a model which can be described as follows (cf. Edman & Jóhannesson 1976; Bassingthwaite, Fry & McGuigan 1976; Wohlfart 1979). The amount of calcium in a membrane compartment, at the moment of excitation, is believed to adversely determine the action potential duration. When the extracellular calcium concentration is increased (Fig. 1) more calcium is taken up by the cell leading to a greater force production. At the same time the action potential duration is reduced because of the higher contents of calcium in the membrane compartment. Increasing the stimulation frequency (Fig. 2) represents another way of loading the cardiac cell

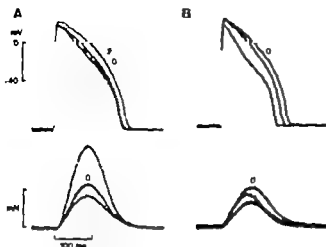


Fig. 4. Oscilloscope records of action potential and isometric myograms from the same experiment as that demonstrated in Fig. 3. The external calcium concentration was 2.0 mM. The recordings from the two test contractions (designated 1 and 2) are superimposed on a control record (designated 0). The first test interval was 0.3 s in panel A and 8.0 s in panel B.

The greatest changes in force production occur in the second test contraction with this stimulation sequence. Fig. 3B (open symbols) shows that peak force of the second test contraction (F_2) is similarly related to the interval as AP_1 and thus reduced for the longer intervals.

Fig. 4 exemplifies original records of action potentials and isometric myograms of the two test contractions. For comparison the records are superimposed on the control traces. It can be seen that peak force of the first test contraction (F_1) is reduced both after a shortened (Fig. 4A) and a lengthened (Fig. 4B) test interval. The inverse relationship between peak isometric force and action potential duration is thus not seen in contraction 1 where the nearest preceding excitation interval is varied.

2 The effects of low calcium concentrations on the relation between action potential and the preceding interval

The curve relating the action potential duration of the first test contraction (AP_1) to the preceding interval (Fig. 3A) was shifted towards longer action potentials when the external calcium concentration was reduced from 2.0 mM to 1.0 mM and 0.5 mM. This is in accordance with the results shown in Fig. 1. However, after the very short (about 0.2 s) and after the very long (about 8.0 s) intervals there was no significant difference be-

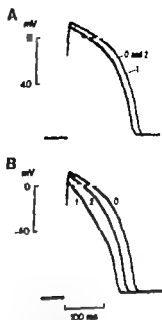


Fig. 5. Oscilloscope records of action potential and isometric myograms from the same experiment as that in Fig. 3 and 4. The external calcium concentration was 0.5 mM. Numbers refer to same pacing sequence as before (Fig. 3A, inset). The test interval was 0.3 s in panel A and 8.0 s in panel B. Note that the changes in action potential duration are as those seen in Fig. 4.

tween the curves in the different calcium concentrations. The well known negative isotropic effect of lowering the external calcium concentration is seen in Fig. 3B where peak force of the second contraction (F_2) has been related to the test interval.

Fig. 5 shows original records of action potentials in an external calcium concentration of 0.5 mM. It can be seen that the effects of varying the interval are similar to those seen in a calcium concentration of 2.0 mM (cf. Fig. 4). Thus the action potential of the first test contraction was shorter in duration after a short interval (Fig. 5A) and reduced after a long interval (Fig. 5B). When calcium concentration was reduced below 0.5 mM the force production was very much reduced and the effect was not completely reversible. These experiments were therefore discarded.

3 The effects of D600 on the action potential duration

The agent D600 is known to block the slow inward current (Kohlhardt et al. 1972; Kass & Tsien, 1977; Nawrath et al. 1977) without any substantial effect on the duration of the action potential. A concentration of 4 μ M was used in the present experiments. This concentration reduced

- R. G. W. Jr & REUTER, H. 1970a. Voltage experiments on ventricular myocardial fibres. *J. Physiol. (Lond.)* 207: 165-190.
- R. G. W. Jr & REUTER, H. 1970b. Membrane ion currents in ventricular myocardial fibres. *J. Physiol. (Lond.)* 207: 191-209.
- T. M. R. 1978. An analysis of the effect of the rate limitation and adrenaline on the duration of the action potential. *Pflügers Arch.* 377: 155-166.
- T. M. R. & JEWELL, B. R. 1978. A study of the ions responsible for rate-dependent shortening of action potential in mammalian ventricular muscle. *J. (Lond.)* 285: 359-380.
- U. S. & ENDO, M. 1968. Calcium ion and muscle traction. *Prog. Biophys. Mol. Biol.* 18: 123-183.
- V. K. A. P. & JOHANSSON, M. 1976. The conduction state of rabbit papillary muscle in relation to action frequency. *J. Physiol. (Lond.)* 254: 365-381.
- NDS, R. E., GREENSPAN, K. & FISCH, C. S. Electrophysiological correlates of contractile type in mammalian and amphibian myocardium. *Circulat. Res.* 3: 252-260.
- ARD, H. A. 1977. Heart: Excitation-contraction coupling. *Ann. Rev. Physiol.* 39: 201-220.
- ES, L. S. & REUTER, H. 1974. Slow recovery from inactivation of inward currents in mammalian myocardial fibres. *J. Physiol. (Lond.)* 40: 703-714.
- SWIRTH, D. NOBLE, D. & TSIEN, R. W. 1972. Dependence of plateau currents in cardiac purkinje fibres on the interval between action potentials. *J. Physiol. (Lond.)* 222: 27-49.
- BERG, G. 1975. Is potassium conductance of cardiac Purkinje fibres controlled by $(Ca^{2+})_i$? *Nature* 3: 273-274.
- S. R. S. & TSIEN, R. W. 1975. Multiple effects of ionic antagonists on plateau currents in cardiac Purkinje fibres. *J. Gen. Physiol.* 66: 169-192.
- S. R. S. & TSIEN, R. W. 1976. Control of action potential duration by calcium ions in cardiac Purkinje fibres. *J. Gen. Physiol.* 67: 599-617.
- LEHARDT, M., BAUER, B., KRAUSE, H. & LECKENSTEIN, A. 1972. Differentiation of the membrane Na and Ca channels in mammalian cardiac fibres by the use of specific inhibitors. *Pflügers Arch.* 325: 309-322.
- LEWARTOWSKI, B., PROKOPCZUK, A. & PYT KOWSKI, B. 1978. Effect of inhibitors of slow calcium current on rested state contraction of papillary muscles and post rest contractions of atrial muscle of the cat and rabbit hearts. *Pflügers Arch.* 377: 167-175.
- MCDONALD, T. F. & TRAUTWEIN, W. 1978. Membrane currents in cat myocardium. Separation of inward and outward components. *J. Physiol. (Lond.)* 274: 193-216.
- MCDONALD, T. F. & TRAUTWEIN, W. 1978b. The potassium current underlying delayed rectification in cat ventricular muscle. *J. Physiol. (Lond.)* 274: 17-246.
- NAWRATH, H., TENEICK, R. E., MCDONALD, T. F. & TRAUTWEIN, W. 1977. On the mechanism underlying the action of d-600 on slow inward current and tension in mammalian myocardium. *Circulat. Res.* 40: 408-414.
- NEW, W. & TRAUTWEIN, W. 1977. Inward membrane currents in mammalian myocardium. *Pflügers Arch.* 334: 1-3.
- NEW, W. & TRAUTWEIN, W. 1977b. The ionic nature of slow inward current and its relation to contraction. *Pflügers Arch.* 334: 24-34.
- NOBLE, D. 1975. The initiation of the heartbeat. Clarendon Press, Oxford.
- REITER, M. & STICKEL, F. J. 1968. Der Einfluss der Kontraktionsfrequenz auf das Aktionspotential des Meerschweinchen-Papillarmuskels. *Naunyn-Schmiedeberg Arch. Pharmacol.* 260: 342-365.
- TRAUTWEIN, W. 1973. Membrane currents in cardiac muscle fibres. *Physiol. Rev.* 53: 793-835.
- TRAUTWEIN, W. & DUDEL, J. 1954. Aktionspotential und Mechanogramm des Warmblüterherzmuskels bei Perioden der Schlagfrequenz. *Pflügers Arch.* 260: 24-39.
- WOHLFART, B. 1979. Relationships between peak force, action potential duration and relaxation interval in rabbit myocardium. *Acta Physiol. Scand.* 106: 395-409.

with calcium. It should be noted however that changes in stimulation frequency will also affect the action potential duration via factor (?) i.e. the length of the preceding interval. The influence from this factor can be kept constant by using the protocol described for Fig. 2. Peak force and action potential duration are here related in a test contraction that is elicited at a fixed interval after a priming period with different stimulation frequencies. The figure shows that the increased inotropic state provided by raising the stimulation frequency is associated with shortened action potentials.

In order to explain the influence of the preceding stimulation interval per se upon the action potential duration, additional assumptions have to be made. (1) Calcium is released from the membrane compartment during the action potential. (?) The membrane compartment is gradually replenished by calcium during the interval between action potentials. An action potential elicited after a long stimulation interval (Fig. 3A and 4B) will therefore be of a short duration. The abbreviated action potentials which are seen after very short intervals (shorter than 0.3 s in Fig. 3A) may be explained by an overriding influence of a slow recovery from inactivation of the slow inward current (Gettes & Reuter 1974) and/or by a persisting outward potassium current (Hauswirth, Noble & Tsien 1977).

The proposed model is compatible with the findings reported here. Lowering the external calcium concentration from 2.0 mM to 1.0 mM and 0.5 mM changed the relation between action potential duration and the preceding interval (Fig. 3A) towards longer action potentials. The curves in Fig. 3A seem to meet, however, for the very short (about 0.2 s) and the very long (about 8.0 s) intervals. In the former case this may be due to the refractoriness of the ion mechanisms behind the action potentials discussed above, as the interval is shortened below 0.3 s. Action potentials elicited after the very long intervals may be independent of the external calcium concentration because the membrane compartment has been gradually loaded with calcium towards the same final filling level.

The compound D600 has been reported to act on the cell membrane and thereby inhibiting the slow inward current (Kohlhardt et al. 1972; Kass & Tsien 1975; Nawrath et al. 1977). This would result in a reduced cellular uptake of calcium and a negative inotropic effect. The duration of the action potential is, however, not affected to any great extent. It has

been shown in the present study that D600 changes the action potential constant in duration over a range of preceding test intervals (Fig. 4). The effect of D600 does not seem to be a consequence of a reduction of activator calcium in the cell, as the relation between action potential duration and preceding interval (Fig. 3A) was still present after lowering the calcium concentration in the solution. In terms of the presented model, it is reasonable that D600 inhibits the coupling between the membrane compartment and the action potential duration. High external calcium concentrations have been shown to neutralize the inhibitor slow inward current by D600 (Kohlhardt 1977). The reduction of the action potential duration that was seen after the long stimulation intervals in the presence of D600 (Fig. 6), may be due to increased calcium contents in the membrane compartment which overcomes the inhibition by D600 (cf. Lewartowski, Protoczek & Kowalski 1978). The reduction of the action potential duration after the short intervals may (as above) be attributed to the action potential elicited in the relative refractory period.

The action potential duration and inotropy of the muscle seem to be mutually related. An increase in the action potential duration is assumed to increase the cellular uptake of calcium and therefore raise the inotropic state of the muscle (Antoni, Jacob & Kaufmann 1969; Wood, R. & Wendmann 1969; Braveny & Sumner 1971). Increased contents of cellular calcium, on the other hand, will shorten the action potential. In this intracellular calcium controls both the contractile force and the action potential duration, this mechanism may serve to stabilize the contractile state of the muscle.

The authors wish to thank Professor K. A. P. Edberg for helpful discussions and for criticism of the manuscript, and Dr M. M. Noble for correcting the manuscript and drawing of the pictures by Miss Eva Hansen. We appreciate the work was supported by grants from the Swedish Medical Research Council (project 15184) from the Medical Faculty of the University of Lund and from the Icelandic Science Foundation.

REFERENCES

- BASSINGTHWAIGHTE, J. B. & TRY, C. M. 1976 Relationship between external calcium and outward current in mammalian atrial muscle: a mechanism for the control of action potential duration. *Journal of Physiology* (Lond) 261, 1-15.

Distribution of blood flow in the dog kidney

Saturation rates of inert diffusible tracers versus uptake of 15 μ m microspheres during vasodilation and vasoconstriction

USEN A. HOPE, A. KIRKEBO, I. TYSSEBOTN and K. AUKLAND

Physiology, University of Bergen, Norway

CLAUSEN G. HOPE, A. KIRKEBO, A. TYSSEBOTN, I. & AUKLAND, K. Distribution of blood flow in the dog kidney. II. Saturation rates of inert diffusible tracers versus uptake of 15 μ m microspheres during vasodilation and vasoconstriction. *Acta Physiol Scand* 1980; 110: 49-58. Received 24 Jan 1980. ISSN 0001-6772. Institute of Physiology, University of Bergen, Norway.

While 15 μ m microspheres (M) in principle provide a measure of glomerular flow, uptake rate of inert diffusible tracers indicates effective or net renal flow, i.e. essentially postglomerular capillary flow. Paired measurements of glomerular and postglomerular flow are made in tissue samples from outer, middle and inner cortex (C₁, C₂, C₃) and juxtaglomerular zones. After control M injection, renal vascular conductance (RVC) was altered in one kidney whereupon a second M injection was made, immediately followed by infusion of the ¹²⁵I-iodoantipyrine (AI) and distilled water. RVC was increased maximally by a infusion of acetylcholine with and without reduced renal arterial pressure, whereas moderate vasodilation was produced by lowering renal arterial or raising arterial pressure. RVC was reduced by LA or infusion of angiotensin II. Within mean RVC range of 50-180% of control the fractional distribution of zonal postglomerular flow remained unaltered, in agreement with previous results obtained from local H₂ gas deaeration rate. Glomerular flow was about 20% higher in C₁ equal in C₂ and 40% lower in C₃ as compared to postglomerular flow in control kidneys. This disparity nearly disappeared during maximal vasodilation and tended to increase during vasoconstriction. The results might suggest variable net postglomerular effective flow in radial direction through the renal cortex. Alternatively the fractional redistribution of M might reflect variable degree of M's slumping at the afferent arterioles along the interlobular arteries.

Key words: Renal blood flow, kidney circulation, local flow measurement, microspheres, inert diffusible tracers, vasodilation, vasoconstriction.

Distribution of renal blood flow among outer and inner cortical layers has been the subject of a number of studies. Several recent reviews (Stern 1975, Stern 1976, Aukland 1976, 1980, Aukland et al. 1977) leave no doubt that methodological differences and inadequacies have muddled the issue—and still do. The present work describes paired measurements by two of the most commonly used methods, both with a record of reproducibility but known to give different results in the dog kidney.

Renal vasodilation is induced by lowering arterial pressure, raising arterial or renal venous pressure or by infusion of vasodilator sub-

stances. 15 μ m microspheres (M) invariably indicate markedly greater increase of vascular conductance in inner than in outer cortex. On the other hand, no such redistribution has been demonstrated in comparable studies with methods based on local uptake or washout rate of inert diffusible flow indicators (DI) in anatomically well defined cortical zones. During renal vasoconstriction induced by occlusion of the carotid arteries, M's indicate a greater fall of conductance in inner than in outer cortex (Banka 1975, Prossnitz 1977), but vasoconstrictor substances do not seem to produce a similar effect. The DI techniques have consistently failed to demonstrate fractional redistribution of renal blood

Distribution of blood flow in the dog kidney

Uptake rates of inert diffusible tracers versus uptake of $15\ \mu\text{m}$ microspheres during vasodilation and vasoconstriction

USEN A. HOPE, A. KIRKEBO, I. TYSSEBOTN and K. AUKLAND

Physiology, University of Bergen, Norway

CLAUSEN G., HOPE, A., KIRKEBO, A., TYSSEBOTN, I. & AUKLAND, K.. Distribution of blood flow in the dog kidney. II. Saturation rates of inert diffusible tracers versus uptake of $15\ \mu\text{m}$ microspheres during vasodilation and vasoconstriction. *Acta Physiol Scand* 1980 110: 249-258. Received 24 Jan. 1980. ISSN 0001-6772. Institute of Physiology, University of Bergen, Norway.

While $15\ \mu\text{m}$ microspheres (Ms) in principle provide a measure of glomerular flow, uptake rates of inert diffusible tracers indicates effective or "arteriole" flow i.e. essentially postglomerular capillary flow. Paired measurements of glomerular and postglomerular flow were made in tissue samples from outer, middle and inner cortex (C_1 , C_2 , C_3) and medullary zones. After control Ms injection, renal vascular conductance (RVC) was altered in one kidney; hereupon second Ms injection was made immediately followed by infusion of the ^{125}I -sodiumthymine and distilled water. RVC was increased maximally by i.a. infusion of acetylcholine with and without reduced renal arterial pressure, whereas moderate vasodilation was produced by low-dose renal arterial or rising arterial pressure. RVC was reduced by i.a. or infusion of angiotensin II. Within mean RVC range of 50 to 180% of control the fractional distribution of renal postglomerular flow remained unaltered, in agreement with previous results obtained from local H_2 gas desaturation rate. Glomerular flow was about 20% higher in C_1 equal in C_2 and 40% lower in C_3 as compared to postglomerular flow in control kidney. This disparity nearly disappeared during maximal vasodilation and tended to increase during vasoconstriction. The results might suggest variable net postglomerular effective flow in radial direction through the renal cortex. Alternatively the fractional redistribution of Ms might reflect variable degree of Ms skimming at the efferent arteriole anastomoses along the interlobular arteries.

Key words: Renal blood flow, kidney circulation, local flow measurement, microspheres, inert diffusible tracers, vasodilation, vasoconstriction.

Distribution of renal blood flow among outer and inner cortical layers has been the subject of a number of studies. Several recent reviews (e.g. 1975, Stern 1976, Aukland 1976, 1980, and in al. 1977) leave no doubt that physiological differences and inadequacies have led the issue—and still do. The present work uses paired measurements by two of the most commonly used methods, both with a record of reproducibility but known to give different results in the dog kidney.

When renal vasodilation is induced by lowering arterial pressure, raising arterial or renal venous pressure or by infusion of vasodilator sub-

stances, $15\ \mu\text{m}$ microspheres (Ms) invariably indicate a markedly greater increase of vascular conductance in inner than in outer cortex. On the other hand, no such redistribution has been demonstrated in comparable studies with methods based on local uptake or washout rate of inert diffusible flow indicators (DI) in anatomically well defined cortical zones. During renal vasoconstriction induced by occlusion of the carotid arteries, Ms indicate greater fall of conductance in inner than in outer cortex (Barik 1975, Prosnitz 1977) but vasoconstrictor substances do not seem to produce a similar effect. The DI techniques have consistently failed to demonstrate fractional redistribution of renal blood

flow among well defined cortical zones during vasoconstriction. Thus local blood flow estimated by M_s and by DI would appear to respond differently to changes in renal vascular conductance. However, failure to detect fractional flow redistribution during vasodilation or constriction might at least in part be ascribed to the methodological inadequacies of previously employed DI techniques as reviewed by Aukland (1976, 1980b). For example, in the case of the local H_2 gas washout technique tissue trauma induced by electrode insertion might cause erroneous measurements. In any case, comparison of M_s and DI results from different laboratories seems rather hazardous due to varying definitions of renal zones, varying M_s sizes, and to inherent basic differences between various DI techniques. However, there can be no doubt that M_s and DI do differ with respect to local flow in outer and inner cortical zones during control conditions as directly demonstrated by paired measurements (Clausen et al. 1979a).

Whereas M_s are extracted from the blood stream by the glomeruli uptake or washout of ^{125}I takes place mainly through the peritubular network of exchange vessels. Thus the effective or nutrient flow with respect to the particular DI used gives a measure of postglomerular capillary flow. Since this capillary network is a low pressure vascular compartment (~ 13 mmHg) minute changes in its interzonal resistance or pressure relations might alter the relationship between the glomerular and postglomerular flow pattern and possibly affect renal excretory function. We therefore designed the present experiments to obtain paired comparison of local flow estimated from uptake rate of two DI and from ^{125}I M_s uptake in zonal tissue samples during experimental conditions known to alter intra-renal M_s distribution.

Preliminary reports of parts of this study have been published (Clausen et al. 1978, Aukland 1978).

MATERIAL AND METHODS

The experiments were made on 45 mongrel dogs of 15–35 kg b.wt. fasted over night but with free access to water. Anesthesia was induced by i.v. injection of Mebumal 35 mg/kg b.wt. and maintained by doses of 3–5 mg/kg as needed. Free airways were maintained by an endotracheal tube without assistance of ventilation. Isotonic saline was infused i.v. at a rate of 2–4 ml/min to maintain a positive water and salt balance. Lower aortic blood pressure (AP) was recorded with Hewlett Packard transducer and re-

corded. One or both kidneys were exposed tonically and nylon snares loosely placed on pedicles. Renal blood flow (RBF) was measured magnetically with a N_2 cotton probe on the renal artery of the experimental kidney. Radiolabelled ^{125}I into the left ventricle through a catheter inserted into the right carotid artery. Reference blood sample was taken at a rate of 1 ml/min from an aortic catheter in the femoral artery to the level of the renal stenosis prior to and lasting 45–60 s after completion of M_s injection. The first M_s injection, M_{s1} , was made during AP and AP control conditions. Then vasodilation or constriction was produced by procedures described below. After 3–5 min with steady RBF and AP the second injection, M_{s2} , was made. Within 3 min after injection, constant rate i.v. infusion of radiolabelled flow indicators (DI) lasting for 8–18 s was made. Serial arterial blood sampling from the aortic catheter, renal pedicles were occluded by tightening the snares before ending the DI infusion. The samples were rapidly excised, frozen in CO_2 -ethanol at $-70^\circ C$ until dissection.

From a 3–7 mm thick sagittal slice cut by an 18-gauge needle, about 10 radial sections were taken. Each sector was divided into three cortical zones (thickness C_1 , C_2 , C_3) and two outer medullary (OM₁ and OM₂). Details on renal identification, counting of radioactivity in tissue and blood samples have been described in a previous paper (Clausen et al. 1979a).

The M_s batches had average diameters of 13.0 (S.D. 1.5) to 16.1 (S.D. 4) μm , as measured microscopically and were labelled with ^{125}I and ^{125}I (Amersham). However, the mean diameter of M_s and ^{125}I were equal in each experiment. The diffusible ^{125}I was mixed in 15 to 30 ml isotonic saline. ^{125}I radioactivity (1 μCi) and 50 μCi of ^{125}I (TlO) and infused at a constant rate of about 1 μCi .

Calculation of local flow by DI was made according to the method of Kety (1951). For details on presentation of the methods, see Hope et al. (1979a, 1979b, 1980). Vasodilation or vasoconstriction and without altered renal perfusion pressure as according to the following protocol.

Group 1 $n=10$ After M_{s1} injection, renal (Ach) was infused at a constant rate into one renal artery through a thin polyvinyl tube inserted retroperitoneally into the blood stream. The infusion rate was adjusted to 50 to 100% increase of RBF before making the M_{s2} infusions.

Group 2 $n=6$ Ach was infused as in group 1. After M_{s1} injection, RBF was thereafter reduced to a level below Ach level by means of an adjustable clamp on the renal artery before the M_{s2} and DI infusions. Renal pressure (RAP) was recorded via a cannula inserted into the renal arterial catheter and was not affected by present volume infusion rates.

Group 3 $n=1$ After M_{s1} injection, superior (Ang) was infused into the renal artery ($n=1$) or femoral vein ($n=6$) at rates adjusted to give a decrease of RBF before the M_{s2} and DI infusions.

Ach and Ang. di. dissolved in isotonic saline in 1 ml at rates of 5–25 μg and 0.1–1.0 $\mu g/min$.

Renal vascular conductance (RVC) calculated from renal perfusion pressure and renal blood flow by microspheres (M_s) and diffusible flow indicators (DI) during vasodilation by acetilcholine reduced renal arterial pressure (RAP) and elevated arterial pressure (UP) and during vasoconstrictor angiotensin II (Ang) and thence elevated arterial pressure (AP)

4 kidneys, E: expl. kidneys

Control period		Exptl. period		RVC ($\mu\text{l}/\text{min}$ g mmHg)	
Pressures (mmHg)	RVC ($\mu\text{l}/\text{min}$ g mmHg)	Pressures (mmHg)		M_s	DI
M_s	M_s	M_s	DI		
AP 129 \pm 17	28.8	126 \pm 18	123 \pm 16	31.2	31.0
AP 129 \pm 17	29.4	126 \pm 18	123 \pm 16	52.5	50.6
AP 136 \pm 17	24.9	Ach	134 \pm 19	17.3	15.7
RAP 134 \pm 17	47.9*		58 \pm 6	49.0	51.1
AP 129 \pm 19	31.4	Ang		1.8	24.3
AP 137 \pm 12	27.6	Ang		9.8	11
AP 142 \pm 14	26.7	RAP ↓	145 \pm 15	23.8	25.1
RAP 133 \pm 17	25.9		75 \pm 7	43.5	50.4
AP 125 \pm 14	25.3	UP ↑	127 \pm 14	24.4	25.6
AP 125 \pm 14	23.5		98 \pm 18*	35.8	35.8

*stat. during control and exptl. periods

mean pressures calculated as AP = (UP + 20) mmHg with UP = 49–17 mmHg.

intravenous infusion rate of Ang was 5–100 μg

were obtained by elevating UP was taken to be UP minus 20 mmHg

14–19 After M_s injection, renal arterial pressure lowered to 60–80 mmHg by an adjustable clamp on artery before the M_s and DI infusions

20–27 After M_s injection, arterial pressure is raised to about 40 mmHg by elevating the catheter further inserted into the renal pelvis via the upper

renal local blood flow (RBF) infusion g was calculated from renal flow rates and the respective zonal flow taken to be C 29 C 23 C₁ 18, OM 12, OM 8 SE 1 kidney eight (Charnick *et al.* 1960). The electrically recorded RBF served only as indicator for changes and to make sure that steady flow was obtained during tracer administration (correct zero flow flow exact probe calibrations)

Renal flow measured with M_s includes flow perfusing the vasa recta and the medullary capillary flow distribution is therefore preselected fraction (A) of total RBF obtained in C₁, C₂ and C₃ by medulla (C₃M) zones. Zonal fractions obtained μ and M_s were compared by Student's *t*-test for 4 data. With DI, here only one measurement was made in each kidney the zonal flow fractions of experimental and control kidneys were compared by *t*-test for 4 data. The data are presented as mean \pm S.D. * P < 0.05 considered significant.

Renal vascular conductance (RVC) was calculated as (AP $\mu\text{l}/\text{min}$ g mmHg) in groups 1 and 3 or as (RBF) in groups 2 and 4. The reduction of perfusion pres-

RESULTS

Average mean arterial pressure (AP) did not differ significantly among the groups during control conditions (Table 1). No consistent changes of AP were observed during the experimental interventions except during infusion of Ang. Mean renal arterial pressure (RAP) recorded during control was 7 (0–20) mmHg lower than AP measured in the lower aorta. During constriction of the renal artery (groups 2 and 4), RAP remained fairly stable but was on average 2.5 (+9 to -6) mmHg lower during DI as compared to M_s infusion the difference being statistically not significant.

Individual results are presented for group 1 vasodilation by Ach only. Fig. 1 demonstrates proportionality between local flow in the zones and RBF with the two DI in control and experimental kidneys over wide range of RBF. With M_s and M_s in experimental kidneys highly significant linear correlations were obtained between zonal flow rates and RBF. In contrast to DI the M_s showed that C flow increased relatively less. C₃M flow relatively

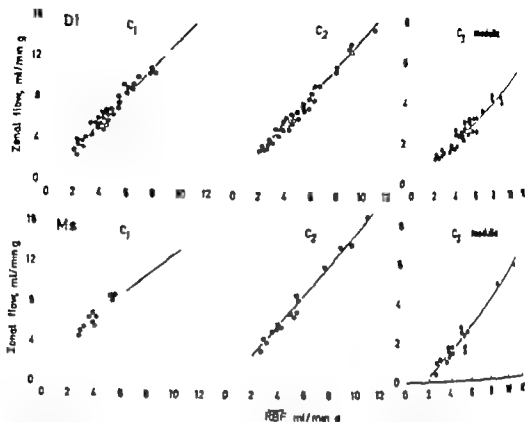


Fig. 1. Local flow with diffusible indicators (DI) and with microspheres (Ms) related to average renal blood flow in 10 dogs during i.a. Ach infusion. C₁, C₂ and C₃M: Outer middle cortex and inner cortex + medulla. Filled circles: Control and experimental kidneys respectively. The regression equations for local flow (F):

C $F = 1.40 \text{ RBF} + 0.24 \quad r = 0.96$
 C $F = 1.40 \text{ RBF} - 0.5 \quad r = 0.98$
 C₂ $F = 0.50 \text{ RBF} + 0.16 \quad r = 0.88$

Ms $F = 1.09 \text{ RBF} + .30 \quad r = 0.94$
 Ms $F = 1.61 \text{ RBF} - 0.63 \quad r = 0.98$
 Ms $F = 0.68 \text{ RBF} - 1.5 \quad r = 0.96$

more than RBF. However, the relationship between C₃ flow and RBF was closely similar with Ms and DI, as seen from the regression equations of Fig. 1.

The average zonal flow fractions and RBF obtained by IAP and THO in all groups are listed in Table 2. The C₁ fractions were not significantly different with the two indicators. However, the C₂ and C₃ flow fractions were slightly higher with THO on average $1.8 \pm 0.8\%$ of RBF ($P < 0.05$) and the C₁ fractions slightly lower $1.4 \pm 0.6\%$ of RBF ($P < 0.05$). RBF tended to be lower with THO, but the difference was statistically not significant.

The most conspicuous observation was that no significant change of the zonal flow fractions occurred in any group with either of the two diffusible indicators.

During maximal dilation induced by Ach and with RAP lowered to about 60 mmHg, RBF averaged about 2.9 ml/min g. A slightly lower average 2.3

ml/min g was obtained during i.v. infusion with RAP averaging 217 mmHg. Furthermore, average RBF was about 160% higher during i.a. infusion of Ang, while RAP remained at control level in both groups. Thus, neither of either RAP or RBF did not per se alter the fractions of effective blood flow.

Microspheres

Table 3 lists the average zonal flow fractions and RBF obtained by Ms in all groups. Comparing zonal flow fractions with Ms₁ and Ms₂ in control showed no significant differences. Furthermore, average zonal flow fractions and RBF with the experimental kidneys were not significantly different from control values, with the exception of group below. Thus, the reproducibility of the Ms should be satisfactory for detecting

onal fractions of total RBF and average renal blood flow (RBF) determined by two diffusible

outer and middle cortex, C₂M: inner cortex + medulla, C=control kidney

m-iodoantipyrine (% of RBF)			RBF (ml/ min/g)	Titrated water (% of RBF)			RBF (ml/ min/g)
C ₁	C ₂	C ₂ M		C ₁	C ₂	C ₂ M	
42.5 ± 3.3	32.6 ± 1.7	24.9 ± 3.5	3.90 ± 1.3	40.3 ± 3.7	32.9 ± 2.0	26.8 ± 4	3.78 ± 1.8
41.3 ± 3.2	33.3 ± 1.3	25.4 ± 3.0	6.40 ± 2.0	39.8 ± 5.4	32.6 ± 3.0	26.3 ± 4	6.35 ± 2.3
44.2 ± 3.5	32.7 ± 1.4	23.3 ± 3.9	3.32 ± 0.7	41.7 ± 2.1	32.8 ± 0.8	25.6 ± 2.6	1.99 ± 0.7
42.8 ± 3.8	33.8 ± 0.9	4.1 ± 3.7	3.25 ± 0.9	40.0 ± 3.5	33 ± 1.5	26.8 ± 3.0	6.8 ± 0.7
42.5 ± 3.6	33.9 ± 1.4	4.4 ± 4.2	2.69 ± 1.0	40.6 ± 2.5	32.5 ± 2.2	26.9 ± 3.8	2.23 ± 0.7
41.6 ± 3.2	32.7 ± 1.2	25.7 ± 3	3.89 ± 0.9	39.5 ± 3.2	32.6 ± 2.0	27.7 ± 4.1	3.37 ± 0.4
40.6 ± 4.8	32.8 ± 2.0	26.7 ± 5.8	3.65 ± 0.8	39.6 ± 4.8	33.5 ± 1.1	26.9 ± 3.3	3.63 ± 0.9
43.1 ± 3.3	31.7 ± 1.7	25.1 ± 3.3	3.33 ± 1.2	42.1 ± 4.3	30.5 ± 1.7	27.4 ± 3.7	3.18 ± 1.2
44.8 ± 3.3	33.1 ± 1.0	22.3 ± 4.0	3.57 ± 0.7	43 ± 2.8	33.0 ± 2.6	23.8 ± 4.0	3.51 ± 1.0

inner zonal M distribution during vasoconstriction. Total renal vascular flow averaged 77 l with M₁s (Table 1) with no difference among the groups (S.D. 1.0) except for the experimental kidney of group 2 where Ach was used i.a. during M₁s. The M₁s results in experimental kidneys agree well with those by M₂s in group 1 (Table 3) both reflecting the effect of Ach infusion. However group 2 consistently differed markedly with respect to M₂ distribution, even though RVC was in the normal range (Table 1), and average RBF fell from 3.4 to 2.3 (M₂s) contrary to the controls of the group. It should be emphasized that superimposed

posed on Ach infusion, RAP was lowered to well below the autoregulatory range (Table 1) and accordingly that experimental kidney weight averaged 85 (S.D. 8.5)% of control kidney weight.

A consistent observation was that the C₂ flow fraction, closely similar to that obtained with M₁ remained practically unchanged both in control and experimental kidneys in all groups (Table 3). Thus the changes in zonal flow fractions may be illustrated with reasonable accuracy as the changes of the C or C₂M flow fractions. Fig. 1 depicts the relationship between changes in C₂M flow fraction and RVC. During vasodilatation C₂M flow increased proportionately more than RBF while the opposite

Zonal fractions of total RBF and average renal blood flow (RBF) determined by micropheres

outer and middle cortex, C₂M: inner cortex + medulla, C=control kidney E=experimental kidney

Control period, M ₁ s (% of RBF)				(RBF) (ml/ min/g)	Exptl period, M ₁ s (% of RBF)				(RBF) (ml/ min/g)
C ₁	C ₂	C ₂ M			C	C ₂	C ₂ M		
51.4 ± 5.9	32.5 ± 3	16.1 ± 4.7	3.72 ± 1.2	Ach	50.8 ± 7.5	33.3 ± 4.6	16.0 ± 4.8	3.94 ± 1.5	6.6 ± 2.2
51.0 ± 6.6	32.8 ± 3.3	16.2 ± 4.5	3.80 ± 1.0		42.0 ± 4.9	34.8 ± 3.4	23.2 ± 4.8	6.6 ± 2.2	
60.3 ± 6.7	28.7 ± 3.7	11.1 ± 3.4	3.39 ± 1.5		60.0 ± 6.3	29.3 ± 3.9	10.3 ± 2.8	3.32 ± 0.7	
43.3 ± 5.8	35.5 ± 1.5	21.1 ± 5.1	6.51 ± 2.7	Ach RAP ↓	42.5 ± 4.4	37.0 ± 1.2	20.6 ± 4.8	2.84 ± 0.7	2.36 ± 0.9
47.6 ± 5	32.4 ± 2.7	20.0 ± 4.6	3.91 ± 1.3		52.6 ± 6.5	33.9 ± 2.3	13.5 ± 5.4	2.36 ± 0.9	
52 ± 4.7	31.0 ± 1.5	16.8 ± 4.5	3.77 ± 1.3		53.4 ± 5.0	30.2 ± 3.1	16.4 ± 4.4	3.45 ± 1.1	
49.7 ± 4.2	31.4 ± 3.1	18.9 ± 3.8	3.50 ± 1.1	RAP ↓	43.6 ± 4.0	33.8 ± 3.0	22.6 ± 4.9	3.26 ± 0.8	3.10 ± 0.7
49.0 ± 5.2	33.7 ± 1.9	17.3 ± 5.0	3.16 ± 0.6		49.1 ± 4.5	32.6 ± 3.0	18.4 ± 3.5	3.10 ± 0.7	
49.7 ± 6.3	33.4 ± 3.9	17.0 ± 4.3	2.94 ± 0.5		46.7 ± 4.0	35.1 ± 4.0	18.1 ± 3.9	3.49 ± 0.7	

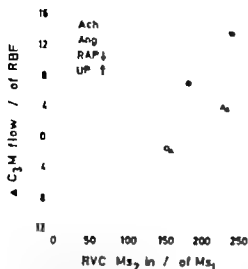


Fig. 2 Changes in the fraction of total RBF perfusing inner cortex and medulla (C_3M) as related to renal vascular conductance (RVC) in experimental kidneys.

pattern was observed during vasoconstriction. The corresponding relative changes in intrarenal resistance blood vessel diameters are -30 to $+25\%$ calculated as the percentage change of $1/RVC$ from Fig. 2 data grouped in 40% RVC intervals.

Comparison of DI and M_s results

Average RBF obtained by the two techniques (Tables 2 and 3) did not differ significantly in any group. Since the C flow fractions obtained by M_s and DI were closely similar in control and experimental kidneys and among the groups relative changes in the flow distribution patterns will be reflected by the respective C/M fractions as summarized in Fig. 3. Vasoconstriction increased the difference whereas vasodilation markedly reduced the difference between the C/M flow fractions obtained by DI and M_s .

Elevation of UP produced a moderate increase of RVC as compared to the other vasodilatory interventions (Table 1). The reduced difference between C/M fractions obtained by M_s and DI was due to a lower C_3M fraction with DI in the experimental kidney ($P < 0.05$) and not to increased C_3M fraction with M_s as in the other vasodilation groups. RBF averaged 3.5 ml/min/g with both M_s and with DI but only 2.9 ml/min/g with M_s in the experimental kidney (Tables 2 and 3). Thus total RBF increased by 19% . However, since left kidney exptl. weight averaged 118 (S.D. 8)% of right kidney weight, RBF did probably not increase during elevation of UP.

DISCUSSION

Flow distribution among cortical zones

The results obtained with two inert diffusers clearly indicate that zonal fractions of C did not change significantly during marked acute decrease of total renal vascular conductance. This agrees well with previous results obtained by local H_2 gas clearance method during vasoconstriction produced by the same experimental means as in the present groups 1 and 2 and during constriction by i.a. infusion of catecholamines (Tyssebohn & Kjerfve 1974, 1979).

Reduction of RAP within the RBF autoregulation range produced no changes in zonal flow fractions with DI. This again agrees with local H_2 clearance studies (Loyning 1971). Elevation of UP produced a slight decline in the C_3M flow fraction. Comparable studies have apparently not been performed with methods based on the uptake or excretion of inert diffusible tracers in well defined renal regions of the dog kidney.

Also the results obtained with $15 \mu m$ latex balloons fairly well with previous observations by using the same technique. Thus a relative increase of deep cortical flow during Ach dilation is reported by McNay & Abe (1970a), Sørensen (1971), Abe et al. (1973a) and Baehler et al. (1977). Rector et al. (1977) observed a 70% locally not significant reduction in the deep cortical flow fraction during vasoconstriction by Ang. Average changes of RVC in these studies are similar to those obtained in the present experiments. Reduction of RAP has been found to increase cortical flow fraction as determined by M_s (Nelson & Abe 1970a, b; Abe et al. 1971a, b). Elevation of UP to 50 – 60 mmHg had the same effect (Björk 1977; Abe et al. 1973b).

A more detailed comparison of the intrarenal flow patterns obtained in the present and previous studies cited above does not seem fruitful due to methodological differences such as the use of 4 cortical zones. Less obvious are the consequences of allowing the kidneys to drain before measuring tracer concentrations and of somewhat different M_s sizes. For example, a 70% volume increase as observed during dilation by Ach (Omvik et al. 1971) would produce a 70% decrease in local H_2 gas clearance. On the other hand, kidneys allowed to drain before dissection are not all M_s sizes.

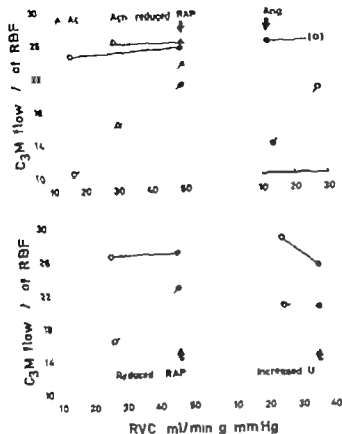


Fig. 3 The fraction of total RBF perfusing outer cortex and medulla (C_3M), as related to renal vascular conductance (RVC). Solid lines: diffusible indicators. Broken lines: microspheres. Open symbols: control kidneys.

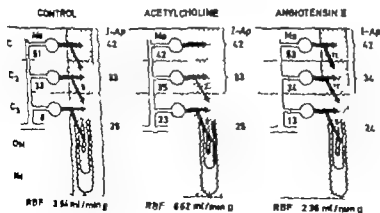


Fig. 4 Hypothetical net inward postglomerular effective flow of blood accounting for the difference between renal flow obtained by microspheres (Me) and diffusible indicator (I-Ap). The numbers give flow in % of total RBF.

cited above may lose 10 to 30% of their volume. As a result local flow per g tissue will be overestimated. Interzonal volume shifts that may occur in the present undrained and rapidly frozen kidneys are probably negligible and M_s and D_I data will be directly comparable. A disproportionate increase of volume in deep renal zones may well occur during elevated UP as suggested by Szabo et al. (1976). This would explain why the C_2M flow fraction fell with D_I and failed to increase with $15 \mu m M_s$ (Fig. 4).

Microspheres somewhat larger than $15 \mu m$ may be subject to purely steric restriction or hindrance from entering afferent arterioles in the dog kidney according to the results of Morkrid et al. (1976). Thus the redistribution of $19 \mu m M_s$ during elevated UP (Bay et al. 1977) could be due partly to increased arteriolar diameter partly to a greater increase of RVC produced by a UP higher than the present average. Similarly steric restriction for $15 \mu m M_s$ during a 70 to 30% reduction of resistance vessel diameter cannot be excluded. It is worth noting that where actually measured in earlier studies the mean M_s diameter turned out to be about 18 or $19 \mu m$ (McNay & Abe 1970a, b; Bay et al. 1977).

With these methodological reservations in mind we conclude that the present results confirm both the previous H_2 gas clearance and M_s studies and directly demonstrate what previously might be inferred from separate studies from different laboratories. $15 \mu m M_s$ may be redistributed among outer and inner cortical zones in spite of maintained distribution of effective or nutrient flow in the dog kidney. Accordingly failure to detect fractional flow distribution between inner and outer cortex by the local H_2 gas washout method cannot be ascribed to local tissue trauma around the inserted electrodes.

Interpretation of the disparate flow distribution patterns obtained with D_I and M_s

It has been repeatedly suggested that redistribution of blood flow among superficial and deep nephrons may be of importance for the regulation of renal salt and water excretion as reviewed by Lameire et al. (1977). However so would probably changes in the relationship between zonal glomerular and post-glomerular flow i.e. between filtering and reabsorbing flow. The present results indicate that

this relationship may be changeable, and variable postglomerular capillary flow of filtration. However this concept rests heavily on the present tracers are valid indicators of glomerular flow (M_s) and postglomerular flow (D_I) as discussed below.

Diffusion of D_I directly from the interlobular arteries (i.l.a.) to C_2 tissue would imply that effective or nutrient blood flow was higher than the blood flow to this zone and might be responsible for the disparity between M_s and D_I observed in control kidneys. However observations made in groups I and II exclude this as a possible explanation. When RBF is reduced, measured D_I concentration difference between i.l.a. blood and C_2 tissue is increased.

Closely similar RVC values were obtained in groups during Ach infusion (Table 1). As RBF was reduced from 6.5 to 2.9 ml/min (1/3) suggesting unaltered i.l.a. diameter, neither diffusion gradient nor surface area through which diffusion might take place were increased, indicating that the absolute amount of D_I up by C_2 directly from i.l.a. was not increased.

Fractional arteriolar inflow to C_2 was less as RBF was reduced as indicated by M_s flow. Thus if an appreciable portion of the D_I was derived directly from the i.l.a. the C_2M fraction should be markedly higher in group I experimental kidneys. Since this is not the case (Table 2) we conclude that direct D_I by diffusion from the deep portion of the i.l.a. is negligible.

The mixing effect of blood flow in the cortex would tend to reduce interzonal D_I concentration differences and accordingly to create an inward effective flow of D_I from C_1 but not from C to C where on average no gradient exists. This also applies to the only inter-cortical inward postglomerular flow of H₂ that taking place in interlobular zones.

Thus neither the mixing effect or inward flow nor direct diffusion from the i.l.a. account for the variable disparity of M_s and D_I flow distribution.

A net inward postglomerular flow in the cortex during control conditions was proposed in previous reports (Clausen et al. 1978, 1979a), as has been the generally accepted concept that $15 \mu m M_s$ is a true estimate of glomerular flow and that D_I

to the tissue essentially through post-glomerular capillaries. Fig. 4 illustrates how this net movement according to the present results during vasodilatation and increases during constriction. This model requires that 70% of renal blood perfusing C glomeruli during conditions attains DI transcapillary diffusions on average 0.5 to 1 mm deeper than at glomerulus. The better agreement between DI observed during vasodilatation and at capillary blood now equilibrates with end drains to the interlobular veins at the end of the parent glomerulus. Thus shift consequence of the disproportionate intravascular conductance in the C arterioles and by the M₂ data. The resulting increase glomerular capillary pressure at C would create a net outward flow of blood through it. We have previously presented a model explaining this effect (Aukland 1978) and provided dependent experimental evidence for a variable cortical postglomerular flow (Aukland

1979) of 15 μ m M₂ at the arteriolar inlet as M₂ a. could be another major—if not the reason for the disparity of the M₂ and DI flow in control kidneys. This is suggested by findings indicating that 15 μ m M₂ underestimates flow by about 20% as compared to simultaneously injected 10 μ m M₂ in the dog kidney (Aukland et al. 1979c). Similar results have been obtained in rats by Yarger et al. (1978) Munro & Bank (1979) and Bank et al. (1979). These findings indicate that the present absolute M₂ to DI ratio in controls might overestimate real zonal flow to postglomerular flow differences. Nevertheless these recent findings may either improve the concept that 15 μ m M₂ truly reflects relative changes of glomerular flow within a zone.

However, it should be emphasized that both M₂ and DI indicated that vascular conductance of the zones increased and decreased during vasodilation and vasoconstriction respectively whereas flow in all renal zones remained in proportion to total RBF with DI this case for C₂ only with M₂. On average M₂ underestimated C₂ flow by about 20% and underestimated C₁ flow by nearly 40% in control kidneys compared to DI. This disparity tended to increase during vasoconstriction, but practically

disappeared during maximal vasodilatation suggesting a variable net postglomerular cortical flow with radial direction.

This work was supported in part by a grant from The Norwegian Research Council for Science and the Humanities.

REFERENCES

- ABE, Y. OKAHARA, T. KISHIMOTO T., YAMAMOTO K. & UEDA, J. 1973 Relationship between intrarenal distribution of blood flow and renin secretion. *Amer J Physiol* 225: 319–323.
- ABE, Y. KISHIMOTO T. YAMAMOTO K. & UEDA, J. 1973 *b* Intrarenal distribution of blood flow during arterial and venous pressure elevation. *Amer J Physiol* 224: 746–751.
- AUKLAND, K. 1978. Renal blood flow. In: Thorau, K., ed. *Kidney and Urinary Tract Physiology II*. Internat. Rev. Physiol. Vol. 11. University Park Press, Baltimore pp. 23–79.
- AUKLAND, K. 1978. Renal hemodynamics. *Proc Internat Congr Nephrol 7th Montreal*, pp. 393–599.
- AUKLAND, K. 1980a. Redistribution of intrarenal blood flow: Facts or methodological artifacts. In: Leaf, A., Orbach, O., Bolis, L. & Gorad, S. eds. *Renal Pathophysiology — Recent advances*. Raven Press, New York pp. 145–154.
- AUKLAND, K. 1980b. Methods for measuring renal blood flow. Total flow and regional distribution. *Ann Rev Physiol* 42: 543–555.
- BAEHLER, R. W. CATANZARO A. J. STEIN J. H. & HUNTER, W. 1973. The radiolabeled frog red blood cell. A new marker of cortical blood flow distribution in the kidney of the dog. *Circulat Res* 32: 716–724.
- BANKS, L. TAN, M. & L. TRINH TRANG & GRUNFELD, J. P. 1979. Measurement of glomerular blood flow in rabbits and rats. Errorless findings with 15- μ m microspheres. *Kidney Internat* 15: 126–133.
- BANKS, R. O. 1975. Intrarenal blood flow distribution. Effect of carotid occlusion. *Proc Soc Exp Biol Med* 150: 327–330.
- BAY, W. H. STEIN J. H. RECTOR, J. B. OSGOOD, R. W. & FERRIS, T. P. 1972. Redistribution of renal cortical blood flow during elevated prearterial pressure. *Amer J Physiol* 222: 33–37.
- CARRIERE, S. 1975. Factors affecting renal cortical blood flow. A review. *Can J Physiol Pharmacol* 53: 1–20.
- CLAUSEN G., HOPE, A. KIRKEBO A., TYS-SEBOM I. & AUKLAND K. 1978. Glomerular versus postglomerular capillary blood flow. *Abstr 1st Congr Nephrol 7th Montreal*, p. F-12.
- CLAUSEN G., HOPE, A. KIRKEBO A., TYS-SEBOM I. & AUKLAND K. 1979. Distribution of blood flow in the dog kidney. I. Saturation rates for most diffusible tracers, ¹²⁵I-iodoantipyrine and tritiated water versus uptake of microspheres under control conditions. *Acta Physiol Scand* 107: 69–82.

- CLAUSEN G, KIRKEBO A, TYSSEBOTN I, OFJORD E. S. & AUKLAND K. 1979b. Erroneous estimates of intrarenal blood flow distribution in the dog with radiolabelled microspheres. *Acta Physiol Scand* 107: 385-387.
- CLAUSEN G, KIRKEBO A, TYSSEBOTN I, OFJORD E. S. & AUKLAND K. 1980. Skinning of microspheres causes false estimates of blood flow distribution in the dog kidney. *Acta Physiol Scand* 108: Abstr. 11A.
- HOPE A, CLAUSEN G & AUKLAND K. 1976. Intrarenal distribution of blood flow in rats determined by 14 C-iodoantipyrine uptake. *Circulat Res* 39: 36-370.
- KETY S. S. 1951. The theory and applications of the exchange of inert gas at the lungs and tissues. *Pharmacol Rev* 3: 1-41.
- LOYNING E. W. 1971. Effect of reduced perfusion pressure on intrarenal distribution of blood flow in dogs. *Acta Physiol Scand* 81: 191-20.
- LAMEIRE N. H., LIFSCHITZ, M. D. & STEIN J. H. 1977. Heterogeneity of nephron function. *Ann Rev Physiol* 39: 159-184.
- McNAY J. L. & ABE, Y. 1970a. Redistribution of cortical blood flow during renal vasodilatation in dogs. *Circulat Res* 27: 103-1032.
- McNAY J. L. & ABE, Y. 1970b. Pressure-dependent heterogeneity of renal cortical blood flow in dogs. *Circulat Res* 27: 571-587.
- MIMRAN A. & CASELLAS D. 1979. Microsphere size and determination of intrarenal blood flow distribution in the rat. *Pflügers Arch* 382: 233-240.
- MORRIS, L., OFSTAD J. & WILLASSEN Y. 1978. Diameter of afferent arterioles during autoregulation estimated from microsphere data in the dog kidney. *Circulat Res* 42: 181-191.
- OMVIAK P. Jr, RAEDER M. & KILPATRICK J. 1960. Mutants of renal cortical volume. *Am J Physiol* 196: 1560-1567.
- PROSNITZ, E. H., ZAMBRASKI, E. J. & DEAN, F. 1977. Mechanism of intrarenal blood flow reduction after carotid artery occlusion. *Am J Physiol* 233(1): F167-F172.
- RECTOR, J. B., STEIN J. H., BAY W. H., OLIVER, R. W. & FERRIS T. F. 1977. Effect of norepinephrine and vasopressor agents on distribution of renal flow. *Am J Physiol* 222: 115-1131.
- STEIN J. H. 1976. The renal circulation. In: *Handbook of Physiology*, Vol. 12, Part 1, eds. M. A. Rector & F. C. eds. The Kidney, Vol. 12, Philadelphia pp. 215-50.
- STEIN J. H., FERRIS, T. F., HUNTER, I., SMITH T. C. & OSGOOD, R. W. 1971. Effect of renal vasodilatation on the distribution of blood flow in the kidney of the dog. *Circulat Res* 29: 1429-1438.
- SZABÓ G., POSCH E., ROSIVALL, L., FUS, A. & HÄRSING L. 1976. Renal blood flow in unilateral obstruction measured with 86 Kr and 86 Rb uptake techniques and with an electromagnetic flowmeter. *Pflügers Arch* 367: 33-36.
- TYSSEBOTN I. & KIRKEBO A. 1979. Effect of vasoactive agents on the distribution of renal blood flow in dogs. *Acta Physiol Scand* 95: 204.
- TYSSEBOTN I. & KIRKEBO A. 1979. Renal blood flow distribution measured by hydrostatic pressure during dopamine and acetylcholine administration. Effect of electrode thickness and position on current. *Acta Physiol Scand* 106: 385-393.
- YARGER, W. E., BOYD, M. A. & SCHRAEDER, W. 1978. Evaluation of methods of measuring glomerular and nutrient blood flow in rat kidney. *Am J Physiol* 235(5): H597-H600.

Effect of β -receptor blockade on adrenaline-induced changes in redox and energy state in m. vastus lateralis and m. soleus of the rat in vivo

FELLENIUS, ROLAND HEDBERG and NILS KARLSSON

Department of Analytical Chemistry and Biochemistry AB Hålsjö, Mölndal, Sweden
Institute of Zoophysiology University of Uppsala, Sweden

FELLENIUS E., HEDBERG, R. & KARLSSON, N. The effects of β -receptor blockade on adrenaline-induced changes in redox and energy state in m. vastus lateralis and m. soleus of the rat in vivo. *Acta Physiol Scand* 1980, 110: 259-266. Received 23 Jan 1980. ISSN 0001-6772. Department of Analytical Chemistry and Biochemistry AB Hålsjö Mölndal and Institute of Zoophysiology University of Uppsala, Sweden.

The metabolic changes in blood red (m. soleus) and white (m. vastus lateralis) skeletal muscle fibres were investigated after short-term (3 min) infusion of adrenaline with or without prior treatment with propranolol or metoprolol. The adrenaline-induced increase in plasma lactate levels was totally prevented by prior treatment with metoprolol or propranolol whilst the β -blockers had no effect on blood glucose levels. Similar effects on lactate levels were found in the m. soleus, while metoprolol was less effective than propranolol in m. vastus lateralis. Adrenaline decreased the level of muscle creatinine phosphate and ADP causing the equilibrium of the creatine kinase reaction to change in the direction of ATP synthesis, although the level of ATP usually decreased. This effect was more pronounced in m. vastus lateralis compared with m. soleus. The $[ATP]/[ADP]$ $[Pi]$ -ratio tended to increase during infusion of adrenaline. This effect was counteracted by metoprolol but not by propranolol. The effects on the 'phosphate potential' ($[ATP]/[ADP]$ $[Pi]$) and the equilibrium within the creatine kinase were more pronounced in m. vastus lateralis than in m. soleus. The results demonstrate the possible role of receptors other than β -receptors, i.e. α -receptors, in mediating changes in plasma glucose levels, while plasma lactate levels are regulated by the β -adrenergic system. The role of β -receptors in mediating changes in muscle lactate levels may differ in m. soleus and in vastus lateralis with relative predominance of β_1 -receptors in m. vastus lateralis. Quantitative and qualitative differences in the adrenergic control of the energy state in the two types of muscle fibres were obvious, although it was not possible to distinguish clearly between the relative importance of α , β_1 and β_2 -receptors.

Key words: Adrenaline, propranolol, metoprolol, redox and energy state, red and white muscles.

Recent investigators have recently shown that β -blockers decrease the time to exhaustion during maximal exercise both in man (Banks et al. 1978, Buskirk et al. 1979) and the experimental animal (Lutz 1977). Muscle fatigue may be one reason for this effect. Other possible explanations are disturbed electrolyte balance (Carlsson et al. 1978) or reduced substrate supply resulting from decreased blood flow and muscle glycogenolysis and adipose tissue lipolysis (Galbo et al. 1977, Fellenius et al. 1979). Lutz (1978) found that glucose infusion restores animals' ability to resume exercise during prolonged treatment.

In order to gain more insight into the possible role of β -adrenergic control in muscle metabolism, the redox and energy state and substrate levels were measured in a glycolytic (superficial portion of m. vastus lateralis) and an oxidative (m. soleus) muscle of the anesthetized rat during β -receptor activation and inhibition. The animals were exposed to the agonist adrenaline for a very short time (3 min). This design is of special relevance in view of the finding that the β -adrenergic system rather than the energy demand appears to play the cardinal role in the mobilization of energy sources during exercise (Lusckwitz 1978).

Table 1 The effect of adrenaline, propranolol and metoprolol on blood levels of glucose, lactate and pyruvate ($\mu\text{mol/g wet wt}$)

The results are means \pm S.D. with the number of observations in parentheses. Details of the experimental conditions are given in Materials and Methods.

Experimental conditions	Glucose	Lactate	Pyruvate	Lactate:pyruvate
Control T=0 min	6.04 ± 0.41 (7)	1.44 ± 0.1 (7)	0.14 ± 0.018 (5)	11.9 ± 9.6 (5)
Saline + saline	4.96 ± 0.1 (7)	0.98 ± 0.70 (7)	0.075 ± 0.01 (7)	13.1 ± 19 (7)
Saline + adrenaline	6.4 ± 0.89 (11)	1.88 ± 0.77 (8)	0.119 ± 0.025 (7)	13.8 ± 8 (7)
Propranolol + saline	5.61 ± 0.67 (6)	0.97 ± 0.09 (6)	0.096 ± 0.007 (6)	9.7 ± 11 (6)
Propranolol + adrenaline	6.89 ± 0.86 (8)	0.93 ± 0.07 (7)	0.070 ± 0.010 (7)	13.5 ± 3 (7)
Metoprolol + saline	5.49 ± 0.30 (6)	0.70 ± 0.11 (6)	0.055 ± 0.018 (6)	13.4 ± 16
Metoprolol + adrenaline	6.35 ± 0.28 (9)	1.03 ± 0.10 (8)	0.074 ± 0.013 (7)	14.1 ± 1 (7)

Statistically significant difference ($P < 0.05$)

S+S vs. S+A	x			
P+S	x	x	x	
P+A	x		x	x
M+S	x			
M+A	x	x		
S+A vs. P+A		x	x	
M+A		x		
P+S vs. P+A	x		x	x
M+S		x	x	x
P+A vs. M+A				
M+S vs. M+A	x	x		

Table 2 The effect of adrenaline, propranolol and metoprolol on tissue metabolite levels of the lateralis ($\mu\text{mol/g wet wt}$)

The results are means \pm S.D. The number of observations is 5 unless otherwise stated in parentheses.

Experimental condition	ATP	ADP	AMP	P	CP
Control T=0 min	5.77 ± 0.36 (4)	0.663 ± 0.073 (4)	0.05 ± 0.006 (4)	13.3 ± 1.4 (4)	13.3 ± 1.4
Saline + saline	5.61 ± 0.64	0.849 ± 0.049	0.03 ± 0.019	13.7 ± 3.1	13.7 ± 3.1
Saline + adrenaline	5.03 ± 0.44 (7)	0.663 ± 0.110 (8)	0.031 ± 0.018	10.8 ± 0.4 (6)	19 ± 4
Saline + propranolol	5.38 ± 0.46	0.707 ± 0.028	0.03 ± 0.007	15.5 ± 1.7	17.1 ± 1.7
Adrenaline + propranolol	4.49 ± 0.60	0.60 ± 0.07	0.026 ± 0.004	9.0 ± 1.6	22 ± 1
Metoprolol + saline	6.22 ± 0.47	0.875 ± 0.085 (7)	0.01 ± 0.014	19.8 ± 1.6	22 ± 1
Metoprolol + adrenaline	4.04 ± 0.4	0.620 ± 0.05	0.029 ± 0.007	1.6 ± 1.5	16 ± 1

Statistically significant difference ($P < 0.05$)

S+S vs. S+A		x			
P+S		x			x
P+A		x			
M+S					x
M+A	x	x		x	x
S+A vs. P+A					
M+A	x	x		x	
P+S vs. P+A		x			x
M+A		x			x
P+A vs. M+A				x	
M+S vs. M+A	x	x		x	

RIALS AND METHODS

Sprague-Dawley rats weighing 200–230 g, pur from Arturimex, Sollefteå, Sweden, were used. were fed ad libitum on a standard animal os, Sodertälje, Sweden and were allowed on to water prior to the exper.

d analytical grade laboratory reagents were ob- two E. Merck AG, Darmstadt, West Germany and coenzymes were obtained from Boehringer Mannheim, West Germany or Sigma Chem- St. Louis, MO, USA. Solutions of hexobarbital (5%) are freshly prepared from Evipan (sodium AG, Leverkusen, West Germany). Propranolol (P) and metoprolol (Soloken) are purchased L. Pharmaceutical Comp. Göteborg, Sweden and ole Pharmaceutical Comp. Mölndal, Sweden, re- tly.

Animal procedure

are anesthetized by an intra-peritoneal injection of rothal (30 mg/kg body wt) and mounted on an op- plate. Skin covering the right hind-leg was re- Fat covering the vastus lateralis was removed and solen was localized by blunt dissection. Saline were used to prevent surface evaporation from the

muscle tissue during the experiment. The abdominal cavi- ty was opened with a midline incision and loose fat cover- ing the inferior vena cava and the ilio-lumbar vessels was removed. Propranolol (7.0 µmol/kg body wt), metoprolol (6.0 µmol/kg body wt) or saline was slowly injected (0.25 ml over 2 min period T=0 min.) into the right ilio-lum- bar vein. At T=25 min. cannula, connected to an infu- sion pump, was placed in the right ilio-lumbar vein for the infusion of adrenaline. At T=30 min the infusion pump was started and adrenaline (33 µmol/kg body wt) or saline was infused over 3 min period. The injected compounds were dissolved in 0.9% NaCl and the total volume of each agent injected was 0.2–0.3 ml. Controls were given saline at T=0 min and at T=30 min and are referred to as saline + saline. The influence of the period of recovery on metabolite levels was also investigated by means of samples taken immediately after the dissection (T=0 min). These experiments are referred to as control, T=0 min.

Sampling of muscle and blood

3 min after the infusion of adrenaline, blood sample (2 ml) was drawn from the inferior vena cava into heparinized syringe. Muscles were then rapidly excised from the right hind-leg and frozen with aluminum tongs cooled in liquid nitrogen (Wolfsberger et al. 1960). The muscles were in- sulated ("red") consisting mainly of slow-twitch oxidative fibres (Amano et al. 1973) and the superficial portion of the vastus lateralis ("white") consisting mainly of fast-twitch glycolytic fibres (Amano et al. 1973, Balci et al. 1972). In some experiments the corresponding muscles from the left hind-leg were used for the determination of glycogen content.

Analytical methods

Blood samples were added to 0.6 M HClO₄. The extracts were neutralized with KOH and the precipitated KClO₄ was removed by centrifugation. The concentrations of lactate, glucose and pyruvate were measured in these extracts as described previously (Fellenius et al. 1973, Karlsson et al. 1973).

HClO₄ extracts of muscle tissue were prepared, neu- tralized and the metabolites analysed as described previ- ously (Fellenius et al. 1973, Karlsson et al. 1973). Muscle tissue was hydrolyzed in 5 M KOH for the analysis of glycogen as described by Pödenphant (1962). Student's *t*-test was used for statistical analysis, *P* < 0.05 being considered statistically significant.

Abbreviations

Adenosine-triphosphate (ATP), adenosine-diphosphate (ADP), adenosine-mono-phosphate (AMP), inorganic phosphate (P_i), creatine-phosphate (CP), creatine (C).

RESULTS

Metabolites in blood

Blood concentrations of glucose, lactate and pyru- vate are shown in Table 1. A statistically significant difference was found in glucose and lactate levels

Lactate	Pyruvate
2.63 ± 0.35 (4)	0.207 ± 0.004 (4)
3.26 ± 0.82 (7)	0.299 ± 0.034 (6)
4.60 ± 0.69	0.278 ± 0.046
1.57 ± 0.77	0.165 ± 0.031
2.17 ± 0.47	0.134 ± 0.018
4.6 ± 0.45	0.233 ± 0.040
3.60 ± 0.40	0.18 ± 0.026

x

Table 3 The effect of adrenaline, propranolol and metoprolol on the red α and energy state of *Crustaceus lateralis*

The results are means \pm S.D. of 5 observations, unless otherwise stated in parentheses. Details of the difference given in the text.

Experimental conditions	[ATP] [ADP][P]	[ATP][C] [ADP][CP]	EC	[ADP] [ATP][AMP]	[Lac] [Pyruv]
Control T=0 min	0.67 \pm 0.1 (4)	0.84 \pm 0.35 (4)	0.938 \pm 0.003 (4)	3.5 \pm 0.54 (4)	13.1
Saline + saline	0.53 \pm 0.4	3.85 \pm 0.43	0.918 \pm 0.017	1.1 \pm 0.74	11.4
Saline + adrenaline	0.65 \pm 0.06 (6)	6.20 \pm 1.59 (6)	0.928 \pm 0.01	10 \pm 0.70	16.3
Propranolol + saline	0.51 \pm 0.13	4.28 \pm 0.57	0.931 \pm 0.006	7.66 \pm 4.42 (4)	9.2
Propranolol + adrenaline	0.85 \pm 0.13	4.90 \pm 0.48	0.97 \pm 0.006	3.1 \pm 0.86	11.13
Metoprolol + saline	0.35 \pm 0.04	3.50 \pm 0.1	0.927 \pm 0.004	8.25 \pm 3.13	16.44
Metoprolol + adrenaline	0.4 \pm 0.08	5.16 \pm 0.68	0.918 \pm 0.005	3.53 \pm 1.04	16.13

Statistically significant difference ($P < 0.05$)

S+S vs. S+A		x			
	P+S			x	
	P+A	x			
	M+S			x	
	M+A	x		x	
S+A vs. P+A					
	M+A	x		x	
P+S vs. P+A	x				x
	M+S	x			
P+A vs. M+A	x				
M+S vs. M+A	x	x	x		x

Table 4 The effect of adrenaline, propranolol and metoprolol on tissue metabolite levels of the α ($\mu\text{mol/g}$ wet wt)

The results are means \pm S.D. The number of observations is 5, unless otherwise stated in parentheses.

Experimental condition	ATP	ADP	AMP	Pi	CP
Control T=0 min	3.66 \pm 0.20 (4)	0.771 \pm 0.038 (4)	0.039 \pm 0.004 (4)	8.8 \pm 0.6 (3)	9.94
Saline + saline	4.39 \pm 0.48 (6)	0.813 \pm 0.107 (6)	0.044 \pm 0.008 (4)	11.0 \pm 0.8	11.64
Saline + adrenaline	3.99 \pm 0.35 (6)	0.736 \pm 0.079 (6)	0.049 \pm 0.011 (6)	8.4 \pm 1.9	10.41
Propranolol + saline	3.67 \pm 0.13	0.717 \pm 0.042	0.028 \pm 0.024	10.1 \pm 0.6	9.84
Propranolol + adrenaline	3.40 \pm 0.3	0.713 \pm 0.066	0.044 \pm 0.007	7.8 \pm 1.0	9.21
Metoprolol + saline	4.3 \pm 0.04	0.815 \pm 0.079	0.026 \pm 0.010	10.3 \pm 0.8	11.51
Metoprolol + adrenaline	3.01 \pm 0.19	0.703 \pm 0.023	0.065 \pm 0.005	10.4 \pm 0.4	9.64

Statistically significant difference ($P < 0.05$)

S+S vs. S+A					x
	P+S	x			
	P+A	x		x	
	M+S				x
	M+A	x	x		
S+A vs. P+A	x				
	M+A	x			
P+S vs. P+A	x			x	x
	M+S	x			
P+A vs. M+A	x			x	
M+S vs. M+A	x	x	x		x

control, $T=0$ min. and the saline + saline infusion of adrenaline caused an increase in blood glucose concentration compared with the control. An increase in blood glucose concentration was also seen when propranolol was given 30 min before the infusion of adrenaline or saline. However the increase in blood glucose after propranolol and saline alone was lower than that after the infusion of adrenaline, with or without pretreatment with a β -blocker. A statistically significant increase in lactate was seen after adrenaline infusion. This increase was abolished when a β -blocker was given 30 min before adrenaline. As compared with control, a decrease in lactate concentration was seen after the administration of metoprolol. There was no change in blood pyruvate levels essentially parallel changes in lactate levels.

Use contents of *m. vastus lateralis*

Concentrations of adenine nucleotides, creatine, creatine phosphate, lactate and pyruvate in muscle are shown in Table 2. The recovery period of 10 min after dissection had itself an effect on all the metabolites. Thus, ADP, CP, lactate and pyruvate levels increased in saline-saline

compared to control $T=0$ min. The infusion of adrenaline caused an increase in lactate levels, while the concentrations of CP and ADP decreased.

The adrenaline-induced increase in lactate was abolished by the β -blockers, propranolol being the more efficient. The changes in CP levels induced by the β -blockers alone differed significantly from those induced by β -blockers plus adrenaline. Similar effects were observed on ADP levels. ATP levels were relatively constant in the experiment with metoprolol + adrenaline, where a decrease was observed compared to metoprolol + saline or saline + saline.

The products $[ADP]/[ATP]$, $[AMP]$ and $[ATP]/[CP]$, $[ADP]/[CP]$ which reflect the equilibrium states of the adenylate kinase and creatine kinase reaction respectively are shown in Table 3 together with the cytosolic redox state ($[lactate]/[pyruvate]$ ratio), the phosphate potential ($[ATP]/[ADP]$, P_i) and the energy charge (EC), which reflects the ratio $[ATP + 1/2 ADP]/[ATP + ADP + AMP]$ (Atkinson 1968). The $[lactate]/[pyruvate]$ ratio showed only small changes. The ratio was about the same in the presence of saline + adrenaline, propranolol + adrenaline and metoprolol + adrenaline. In the experiments with β -blockers alone the ratio tended to decrease compared with those in which both β -blockers and adrenaline were used. Both propranolol and metoprolol tended to increase the ratio $[ADP]/[ATP]$, $[AMP]$. The creatine kinase reaction moved in the direction of ATP synthesis during adrenaline infusion and this effect was not counteracted by the β -blockers. The phosphate potential was little affected by adrenaline infusion unless the animal had been treated with a β -blocker.

Metabolite contents of *m. soleus*

In general, very much smaller quantitative changes in metabolite levels during β -receptor activation or inhibition occurred in the *m. soleus* (Table 4) than in the *m. vastus lateralis*. During the recovery phase, i.e. control $T=0$ min. versus the saline + saline experiment, ATP, P and CP increased statistically significant ($P < 0.05$). Adrenaline infusion had very little effect on metabolite levels. In the presence of metoprolol, adrenaline induced a relatively pronounced drop in the level of ATP while no corresponding significant changes occurred with propranolol. Propranolol alone tended to decrease ATP, CP, lactate and pyruvate levels.

	Lactate	Pyruvate
4	1.38 ± 0.19 (4)	0.131 ± 0.010 (4)
	1.53 ± 0.31	0.080 ± 0.030 (4)
	2.22 ± 0.45	0.135 ± 0.024
	1.12 ± 0.18	0.144 ± 0.014
	1.22 ± 0.32	0.112 ± 0.025
	0.85 ± 0.31	0.155 ± 0.007
	1.35 ± 0.45 (6)	0.089 ± 0.025

Table 5 The effect of adrenalin, propranolol and metoprolol on the redox and energy state of soleus

The results are means \pm S.D. of 5 observations unless otherwise stated in parentheses. Details of the conditions are given in Materials and Methods.

Experimental conditions	[ATP] [ADP][P]	[ATP][C] [ADP][CP]	EC	[ADP] ^a [ATP][AMP]	[Lac] [Pyruv]
Control T=0 min	0.53 \pm 0.07 (3)	4.63 \pm 0.79 (4)	0.891 \pm 0.005 (4)	4.34 \pm 0.40 (4)	81.14
Saline + saline	0.48 \pm 0.04	4.59 \pm 0.33 (4)	0.895 \pm 0.003 (4)	4.76 \pm 0.94	88.2
Saline + adrenalin	0.65 \pm 0.06 (6)	5.37 \pm 0.70	0.900 \pm 0.01 (6)	1.86 \pm 0.43	18.91
Propranolol + saline	0.49 \pm 0.05	4.77 \pm 0.34	0.899 \pm 0.011	4.99 \pm 0.27 (6)	77.4
Propranolol + adrenalin	0.6 \pm 0.11	4.29 \pm 0.44	0.890 \pm 0.006	3.46 \pm 0.64	88.41
Metoprolol + saline	0.53 \pm 0.08	4.73 \pm 0.64	0.903 \pm 0.006	5.76 \pm 0.96	85.1
Metoprolol + adrenalin	0.41 \pm 0.0	4.04 \pm 0.4	0.873 \pm 0.006	5.55 \pm 0.13	86.31

Statistically significant difference ($P < 0.05$)

S+S vs	S+A				x
	P+S				
	P+A	x	x		x
	M+S				
	M+A	x	x		
S+A vs	P+A	x		x	x
	M+A	x	x		
P+S vs	P+A	x	x		x
	M+S				
P+A vs	M+A	x	x	x	
M+S vs	M+A	x	x		x

while metoprolol alone produced statistically significant effects only in lactate and pyruvate levels.

Effects on the [lactate]/[pyruvate] ratio and the [CP] ratio (Table 5) were found during the recovery phase i.e. control T=0 min versus saline/saline. Adrenalin only affected the energy and redox state in the presence of a β -blocker. Thus the phosphate potential was increased by adrenalin after prior treatment with propranolol while the reverse occurred with metoprolol. As compared to the control both β blockers produced a significant lowering of the [lactate]/[pyruvate] ratio. Adrenalin increased the [lactate]/[pyruvate] ratio after prior β receptor blocker treatment.

Effect on glycogen content

Glycogen levels (Table 6) changed very little during the recovery period although a statistically significant decrease in glycogen was observed in m soleus. There were no statistically significant effects on glycogen stores during adrenalin infusion. The effect of adrenalin during β -receptor blockade was complex. After propranolol treatment adrenalin induced an increase in glycogen stores in

soleus but not in vastus lateralis. After metoprolol pretreatment adrenalin caused no effects at all but a slight decrease in glycogen stores was sometimes found in vastus lateralis.

DISCUSSION

The dose of adrenalin was chosen to 70% stimulation in heart rate caused by a maximal dose of the β agonist isoprenaline (Fellenius, unpublished observation). The adrenalin induced increase in plasma glucose levels was not prevented by prior β -receptor blockade. This is in line with the possible mediation of hepatic glucose release by α receptors suggested by Ellis (1979).

The adrenalin induced increase in plasma lactate levels was reversed by both propranolol and metoprolol (Table 1). Similar effects were found in m soleus (Table 4) while metoprolol was less effective than propranolol in m vastus lateralis (Table 2). This would indicate that the role of β receptors in determining muscle lactate level may vary in m soleus and m vastus lateralis with a relative predominance of β_1 -receptors in the m

The effect of adrenaline, propranolol and α on glycogen content in vastus and in soleus

Its are means \pm S.D. of 5 observations unless stated in parentheses. For details of experiments see Materials and Methods

Initial conditions	Glycogen (μ mol glucose/g wet wt)	
	Vastus	Soleus
T=0 min	36 \pm 1.9	33.0 \pm 3.3
solene	36.6 \pm 8.3 (8)	19.7 \pm 1.4
adrenaline	24.7 \pm 1.9	17.6 \pm 2.1
sol + ad	31.8 \pm 4.6	17.5 \pm 2.3 (4)
sol + ad + prop	33.3 \pm 3.2	25.9 \pm 2.5
sol + ad + prop + α	40.4 \pm 5	4.0 \pm 2.0
sol + ad + prop + α + α	31.8 \pm 8.7	25.9 \pm 0.5

with significant difference ($P < 0.05$)

S+A
P+S
P+A
M+S
M+A
P+A
M+A
P+A
M+S
M+A
M+A

A β_1 -receptor influence in the soleus muscle after surprising in view of previous findings relative importance of β_2 -receptor in muscle metabolism (Arnold 1972, Stanton 1972, Kuo et al 1975, Hestmark & Horn 1975). However previous studies did not differentiate between slow and fast fibres. Furthermore the possibility of a respiratory mechanism secondary to β -blockade is considered before the type and population of receptors can be stated. Thus, any decrease in glycogenolysis and consequently in lactate production may be compensated for by an increase in glucose uptake resulting from activation of base by decrease in glucose-6-phosphate. This would restore lactate production and also the level of lactate. The fact that release is unaffected by β_1 -receptor blockade (William-Olson et al. 1979) in contrast to propranolol treatment, may in addition attract the adrenaline induced increase in muscle glycogenolysis.

Adrenaline may also have some α -adrenergic effects. α -receptors may mediate an effect opposite to the β -effect on muscle metabolism (Dynarowicz 1974a,b).

Besides a direct adrenergic control of skeletal muscle metabolism, an indirect control in the form of adrenergic regulation of skeletal muscle blood flow may exist. This would affect the rate of delivery of substrate which is essential for the maintenance of normal energy and redox states. It is well documented that the two β -blockers may influence the peripheral resistance differently (Åblad et al. 1975).

Although it is not possible to evaluate the relative importance of α , β and β_2 -receptors within the different muscle groups, both quantitative and qualitative differences in adrenergic control seem to exist in the two muscle fibres.

REFERENCES

- ÅBLAD B, BORG K, O CARLSSON E, EK L, JOHNSON O, MALMFORSS T & REGÅRDH C-G 1975 A survey of the pharmacological properties of metoprolol in animals and man. *Acta Pharmacol (Kbh.)* 36, Suppl. V 7-23.
- ARIANO III A, ARMSTRONG R D & EDGERTON V R 1973 Hind limb muscle fibre population of five mammals. *J Histochem Cytochem* 21 51-55.
- ARNOLD A 1972 Differentiation of receptors activated by catecholamines. *J Pharmacol Ed Sc* 27 79-100.
- ATKINSON D E 1968 Citrate and the citrate cycle in the regulation of energy metabolism. *Metabolic role of citrate* (ed. T W Goodwin), pp 23-40. Academic Press, London, New York.
- BALDWIN R M, KLINGERFUS G H, TERJUNG R C, MOLÉ E A & HOLLOSZY J D 1972 Respiratory capacity of white, red and intermediate muscle: Adaptive response to exercise. *Am J Physiol* 222 373-378.
- BANKS D C, PATRICK J M & PEARSON S B 1978 The effects of propranolol and metoprolol on exercise responses in normal man. *Brit J Pharmacol Proc British Pharmacol Soc* p 443.
- CARLSSON E, FELLENIUS E, LUNDBORG P & SVENSSON L 1978 β -adrenoceptor blockers, plasma-potassium and exercise. *Lancet* 2 474-475.
- DYNAROWICZ L 1974 Adrenergic regulation of glycogen breakdown in the heart and skeletal muscle. The role of β -adrenergic receptor. *Acta Physiol Pol* 25 145-150.
- DYNAROWICZ, J 1974b Adrenergic regulation of glycogen breakdown in the heart and skeletal muscle. The role of α -adrenergic receptor. *Acta Physiol Pol* 25 151-158.
- ELLIS S 1978 Some explanations for species differences in hyperglycemic response to adrenergic agents. *Life Sc* 22 1229-36.

- FELLENIUS E, BJÖRKROTH U & KIESSLING K H 1973 Metabolic changes induced by ethanol in muscle and liver tissue of the rat in vivo. *Acta Chem Scand* 7: 361-366.
- FELLENIUS E, CARLSSON E, ÅSTRÖM H, LIPSKA M, LUNDBORG P, SVENSSON L, BENGTSSON C & SMITH U 1979 Metabolic effects of β -adrenoceptor blockade during exercise. 4th Int Symp. Biochem Exercise Brussels. Abstract p. 1.
- GALBO H, CHRISTENSEN N J & HOLST J J 1977 Glucose induced decrease in glucagon and epinephrine responses to exercise in man. *J Appl Physiol* 47: 5-30.
- HARRI M N 1977 Effect of prolonged β -blockade on energy metabolism and adrenergic responses in the rat. *Med Biol* 55: 768-76.
- HOSTMARK A T & HORN R S 1975 Sensitivity of the rat diaphragm adrenergic receptors. *Biochem Pharmacol* 4: 985-88.
- ISSEKUTZ B Jr 1978 Role of β -adrenergic receptors in mobilization of energy sources in exercising dogs. *J Appl Physiol* 44: 869-876.
- KARLSSON N, FELLENIUS E & KIESSLING K H 1975 The metabolism of acetate in the perfused hind-quarter of the rat. *Acta Physiol Scand* 93: 391-400.
- KUO S H, KAMAKA J K & LUNDH G 1973 Adrenergic receptor mediated and hyperglycemia and hyperlactacidemia by sympathomimetic amines in the cat. *J Pharmacol Therap* 702: 301-309.
- PFLEIDERER G 1960 Glykolyse und Glucose mit Hexokinase Pyruvatdehydrogenase. In: Methoden der enzym. Analysen (ed. H. U. Bergmeyer). Verlag Chemie Weinheim/Bergstrasse.
- STANTON H C 1978 Selective metabolic-vascular β -receptor adaptation in the rat. *Pharmacodyn* 196: 46-58.
- WILLIAM-OLSSON T., FELLENIUS E & TORP M & SMITH U 1979 Differential responses to β -adrenergic stimuli: propranolol or metoprolol administration. *Acta Scand* 705: 301-306.
- WOLLENBERGER A, RISTAU O & SCHMIDT H 1960 Eine einfache Technik der röntgen. Abkühlung grösserer Gewebestücke. *Pflügers Ges Physiol* 70: 399-411.

Effects of short term and prolonged infusions of somatostatin on endocrine pancreas body weight and food intake in rats

SVEN B. PETERSSON and S. EFENDIĆ

Department of Endocrinology, Karolinska Hospital, Stockholm, and Department of Histology, University of Uppsala, Sweden

LINDEN P. E., PETERSSON S. B. & EFENDIĆ S. Effects of short-term and prolonged infusions of somatostatin on endocrine pancreas body weight and food intake in rats. *Acta Physiol Scand* 1980 110 267-275. Received 25 Jan 1980. ISSN 0001-6772. Department of Endocrinology, Karolinska Hospital, Stockholm, and Department of Histology, University of Uppsala, Sweden.

Infusion of cyclic somatostatin (700 µg/kg/h) for 4 h in rat fed ad libitum suppressed basal insulin but not glucagon release. It was accompanied by hypoglycaemia during the first hour whereas, at the end of the infusion, hyperglycaemia was present. The same dose of somatostatin applied 60 min prior to and during a 30 min load of glucose or arginine significantly inhibited their effects on insulin and glucagon release. In contrast, when this dose of somatostatin was given during a 24 h period by the i.v. route it did not inhibit glucose induced insulin release though circulating somatostatin levels were constantly and markedly elevated. Furthermore, in rats continuously infused with somatostatin for 4 days, no effect was found either on plasma concentrations of glucose, insulin, glucagon, growth hormone and cyclic AMP or on body weight gain, food consumption or water intake. The pancreases of these animals showed normal concentrations of insulin and glucagon and normal nuclear area of D-cells. Our experiments demonstrate that, in short-term experiments in rats, somatostatin influences insulin and glucagon release as well as glucose homeostasis. Furthermore they suggest that during prolonged administration of somatostatin rats develop mechanisms counteracting the effect of the peptide e.g. peripheral tachyphylaxis.

Key words: Somatostatin, endocrine pancreas.

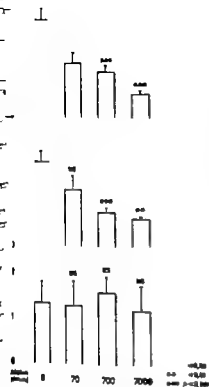
Somatostatin in short-term experiments in man and animals inhibits the release of growth hormone, insulin, glucagon and several gastrointestinal hormones (Efendić et al. 1978). It also decreases intestinal absorption of xylose (Felig & Wahren 1978) and reduces food intake (Lottier & Woods 1978). This would imply that prolonged administration of somatostatin might interfere with blood glucose homeostasis and, in addition, inhibit growth hormone secretion and thus reduce growth hormone secretion of nutrients from the gastrointestinal tract. For this reason we have investigated the effects of short-term and prolonged administration of somatostatin in rats on the functions of the endocrine pancreas, as well as the influence of long-term administration (4-14 days) of the peptide on body weight and food intake.

MATERIAL AND METHODS

Animals. Male Sprague-Dawley rats weighing approximately 200 g were used throughout the experiments. The animals were kept on a complete pellet diet (Ewos Standard Astra Ewos, Stockholm) and water ad libitum. In the experiments dealing with the effect of somatostatin on glucose and arginine stimulated insulin and glucagon release, food was withdrawn at 2 p.m. on the day preceding the experiment. In the studies on the effect of the peptide on basal secretion of pancreatic hormones, the rats had free access to pellet food and to tap water.

Surgical procedure. Short-term infusions of glucose and arginine with or without somatostatin were given under pentothal anaesthesia (sodium pentothal 12.5 mg/100 g of body weight intraperitoneally). To allow blood sampling during the infusions, both external jugular veins were cannulated and short infusion catheters inserted. Blood was collected into chilled heparinized tubes. Plasma was separated and stored until assayed.

- FELLENIUS E, BJÖRKROTH U & KIESSLING K H 1973 Metabolic changes induced by ethanol in muscle and liver tissue of the rat in vivo. *Acta Chem Scand* 27: 2361-2366.
- FELLENIUS E, CARLSSON E, ÅSTRÖM H, LIPSKA M, LUNDBORG P, SVENSSON L, BENGTSSON C & SMITH U 1979 Metabolic effects of β -adrenoceptor blockade during exercise. 4th Int Symp Biochem Exercise Brussels. Abstract p 12.
- GALBO H, CHRISTENSEN N J & HOLST J J 1977 Glucose induced decrease in glucagon and epinephrine responses to exercise in man. *J Appl Physiol* 43: 5-30.
- HARRI M N 1977 Effect of prolonged β -blockade on energy metabolism and adrenergic responses in the rat. *Med Biol* 55: 768-76.
- HÖSTMARK A T & HORN R S 1975 Sensitivity of the rat diaphragm adrenergic receptors. *Biochem Pharmacol* 4: 985-88.
- ISSEKUTZ, B Jr 1978 Role of β -adrenergic receptors in mobilization of energy sources in exercising dogs. *J Appl Physiol* 44: 869-876.
- KARLSSON N, FELLENIUS E & KIESSLING K H 1975 The metabolism of acetate in the perfused hind-quarter of the rat. *Acta Physiol Scand* 93: 391-400.
- KUO S H, KAMAKA J K & LUXELL J 1978 Adrenergic receptor mechanisms involved in hyperglycemia and hyperlactacidemia prior to sympathomimetic amines in the rat. *J Pharmacol Therap* 202: 301-309.
- PFLEIDERER G 196. Glykolytischer und Glucose mit Hexokinase, Pyruvatdehydrogenase. In: *Methoden der enzym. Analysen* (ed H U Bergmeyer), Vol 6 (C Weinheim/Bergstrasse).
- STANTON H C 1978. Selective metabolic and vascular β -receptor antagonists in the rat. *J Pharmacodyn* 196: 46-58.
- WILLIAM-OLSSON T, FELLENIUS E & TORP P & SMITH U 1979 Different metabolic responses to β -adrenergic stimulation: propranolol or isoproterenol administration. *Acta Scand* 205: 201-206.
- WOLLENBERGER A, RISTAU O. & SCHÖG G 1960 Eine einfache Technik der räumlichen Abkühlung grosserer Gewebestücke. *Pflügers Ges Physiol* 270: 399-411.



Effect of somatostatin on insulin, glucagon and responses to arginine. Somatostatin was given 60 min and during 30 min arginine infusion (40 mg/kg/min). The responses are calculated as the difference between values obtained at the end and at the beginning of the arginine infusion (mean \pm S.E.).

tions by means of an ocular screw micrometer (scale 1/200 μ). The largest nuclear diameter was measured as well as the diameter at right angles to it. Six cells with their centres within the sections were selected. The areas of the largest optical cross sections were then calculated in arbitrary units. Synthetic cyclic somatostatin was obtained from the Department of the Kahn Group, Stockholm, and was a gift from AB Vitrax, Stockholm.

TS
of 90 min infusions of somatostatin on induced insulin release and arginine insulin and glucagon release in fasted rats. Insulin and glucagon release during a glucose load (20 mg/kg/min) was significantly reduced by somatostatin dose of 700 ng but not by 70 ng/kg/min

(Fig. 1). The type of interaction of glucose and somatostatin on insulin release was analysed by combining the somatostatin infusion (700 ng/kg/min) with increasing i.v. loads of glucose (5, 10, 20, 30, 60 and 100 mg/kg/min). The insulin response to glucose alone increased with the dose of the glucose but even at such a high glucose load as 100 mg/kg/min. Maximal insulin response was not achieved (Fig. 2). Somatostatin significantly inhibited the insulin response to all glucose doses applied and this effect could not be overcome by the levels of hyperglycemia attained. Therefore in these experiments the interaction of glucose and somatostatin seemed to be non-competitive.

Arginine (40 mg/kg/min) enhanced insulin and glucagon secretion (Fig. 3). Somatostatin at a dose of 70 ng/kg/min. significantly inhibited insulin release. A higher dose of the peptide (700 ng/kg/min) was required for inhibition of glucagon release. The hyperglycemic response to arginine was not modified by somatostatin.

Effects of a 4 h infusion of somatostatin on basal levels of glucose, insulin and glucagon in fed rats. In control animals given saline alone the blood glucose concentrations decreased during the whole experiment (Fig. 4). The lowest glucose levels were observed after 2 h. In the presence of somatostatin (700 ng/kg/min) the blood glucose pattern was altered: after 1 h more marked hypoglycemia was noted, after 2 h there was no change as compared to the controls and finally at 4 h significantly higher blood glucose levels were recorded. Insulin secretion was suppressed during the whole experiment in the somatostatin animals. This inhibition was even more obvious when isobutogenic indices were compared. In contrast, somatostatin only slightly suppressed basal glucagon secretion.

Effects of a 24 h infusion of somatostatin on glucose induced insulin release in fasted rats. In rats infused over 24 h with saline a 30 min infusion of glucose (30 mg/kg/min) applied at the end of experiment induced a marked insulin release (Fig. 5). This effect of glucose was significantly inhibited by somatostatin (700 ng/kg/min) given 60 min prior to and during glucose infusion. In contrast, somatostatin had no significant effect in animals that received the peptide during the whole experiment.

Effects of infusions of somatostatin for 4–14 days on plasma levels of glucose, insulin, glucagon, cyclic AMP and GH on insulin and glucagon content and the number of D-cells in the pancreas as well

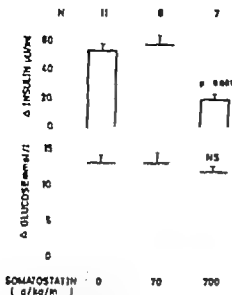


Fig. 1 Effect of somatostatin on insulin and glucose responses to glucose. Somatostatin was given 60 min prior to and during a 30 min infusion of glucose (30 mg/kg/min). The responses were calculated as the differences between values obtained at the end and at the start of glucose infusion (mean \pm S.E.).

In the long term expts. the rats were operated one week before the start of the admin. of somatostatin. At this operation the superior vena cava was cannulated from the external jugular vein. The proximal end of the catheter was brought out through the skin in the midscapular area and held in a protecting harness (Weeks 1971). The catheter was then drawn through a protecting tube connected to a ball-bearing system in the roof of the metabolic cage. This allowed the animal to move freely without damaging the infusion system.

In the 4 h expt. food was withdrawn at p.m. on the day preceding the expt. One group of rats received saline and another somatostatin (700 ng/kg/min) over 4 h. A third group of animals was infused with saline for 0.5 h and thereafter received a 90 min infusion of somatostatin. All animals were anesthetized with penthotal after 0.5 h and the other external jugular vein was catheterized for application of glucose (30 mg/kg/min) during the last 30 min of expt. The same stock solution of somatostatin was prepared and kept at room temperature for 24 h and 90 min infusions.

In the 4 and 14 days expts. somatostatin or saline alone were administered as continuous infusions except for a morning break of one hour. During this break the rats were weighed and the consumed food and water was measured. At the end of expt. in the non-fasted state the rats were killed by decapitation at 8 a.m. and blood was collected for determination of glucose, insulin, glucagon, GH and cyclic AMP. The somatostatin infusion was continued during decapitation and blood sampling. A piece of the pancreas was immediately dissected and frozen in liquid nitrogen while another piece was immersed in Zenker-formalin solution, dehydrated and embedded in paraffin.

Extraction of tissue and hormone assays Pieces of the pancreas were sonicated in appropriate volumes of cold acid ethanol (0.1 M HCl) and extracted for 16–18 h at 4°C . After centrifugation at $3000 \times g$ for 20 min at 4°C the supernatant was removed and stored at -20°C until assayed. The solid extract was diluted with the buffer used for assays. Insulin was determined by a double-antibody immunoassay (Hales & Randle 1963), glucagon by a radioimmunoassay using anti-glucagon (Falkow & Unger 1974), GH by a radioimmunoassay (Edén et al. 1978) and cyclic AMP by a kinase binding assay (Gilman 1970). Glucose was measured by a glucose oxidase method (Nixon 1957). Somatostatin was measured by immunoassay (Efendic et al. 1978b). The antibodies used by ourselves were used at a final dilution of 1:4000. The antigenic specificity of the antibodies was determined using somatostatin analogues (Vernier 1978). Cross-reactivity of the antibody is low with insulin, glucagon, substance P, LH-RH and oxytocin. Somatostatin in plasma was removed by acetone extraction (Armstrong et al. 1970). Insulin index was calculated as the ratio between pre-infusion glucose concentrations.

Quantitative histology of the islets Pancreas from the Zenker-formalin fixed pancreases were impregnated (Heijerström & Hellman 1966). The sections were photographed and the sections revealed with morph's chrome hematoxylin counterstained. Islet (A-cells) were identified by comparing the photomicrographs with the gram-stained sections (Peterson et al. 1970). The nuclear size of the cells was determined by microscopy. In each animal 4 D-cells were measured.

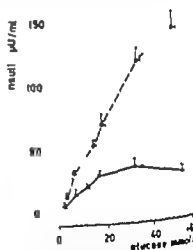
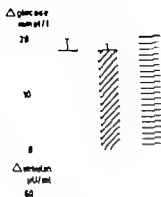


Fig. 2 Interaction of somatostatin and glucose release. Somatostatin (700 ng/kg/min) was given prior to and during a 30 min infusion of glucose (30 , 60 and 100 mg/kg/min). The responses were calculated as the differences between values obtained at the start and at the end of glucose infusions (mean \pm S.E.). Open circles and solid lines denote experiments with somatostatin; open circles and broken lines denote control experiments.

Table 2. *Insulin and glucagon levels in extracts from pancreas (mean \pm S.E.) from rats sacrificed 4 days after the start of the somatostatin infusion*

	Somatostatin (n=11)	Controls (n=11)
Insulin μ U/mg wet weight	1517.0 \pm 199.0	1429.0 \pm 215.0
Glucagon ng/mg wet weight	2.9 \pm 0.6	3.1 \pm 0.4



Effect of somatostatin (700 ng/kg/min) on glucose responses to 30 min infusion of glucose (30 g) in fasted rats. One group of rats received no somatostatin (open bars), and another group received somatostatin (stippled bars) for 22 h and somatostatin for 90 min. The values are calculated as the differences between values at the end and at the start of glucose infusion (S.E.) *** $P < 0.001$. For details see Methods.

DISCUSSION

Recent series of experiments have demonstrated that, in the rat, the derivation of somatostatin infusion is of significance for its action on pancreatic functions. In general, the early effects of the peptide on insulin and

glucagon release present after 4 h of infusion seemed to disappear when the administration was extended over 4 h.

Somatostatin (700 ng/kg/min) was a strong inhibitor of basal insulin release in the present short term experiments. We have previously demonstrated that, in the rat, this dose of the peptide exerted a maximal inhibitory action on basal insulin release (Efendić et al. 1978). In the present study it was demonstrated that the same dose of the peptide also inhibited glucose induced insulin release. It also inhibited arginine induced insulin and glucagon release which confirms previous observations (Gordin et al. 1977; Brown et al. 1976). However basal glucagon release was not suppressed by this dose of the peptide which also is in agreement with the observations of others (Dunbar et al. 1978).

Somatostatin, under prevailing *in vivo* conditions did not inhibit basal glucagon release. Since it strongly inhibited basal insulin an overall hyperglycaemic effect could be expected. This was not the case during the first hour of infusion when somatostatin exerted a marked hyperglycaemic effect. Most likely this was due to interference of the peptide with the intestinal absorption of nutrients. In this context, it is of interest that, in humans, the

Table 3. *Plasma levels of glucose, insulin, growth hormone and cyclic AMP (mean \pm S.E.) 14 days after the start of the somatostatin infusion*

	Somatostatin (n=11)	Controls (n=11)
Glucose mmol/l	7.3 \pm 0.4	6.9 \pm 0.1
Insulin μ U/ml	77.8 \pm 1.7	27.8 \pm 2.6
Growth hormone ng/ml	338.0 \pm 54.0	394.0 \pm 73.0
cAMP pmol/ml	196.0 \pm 97.0	127.5 \pm 56.7

Table 3. *Nuclear area of D-cells (mean \pm S.E.) in pancreas from rats sacrificed 4 and 14 days after the start of the somatostatin infusion*

Days of infusion	Somatostatin	Controls
4	0.5763 \pm 0.0119 (n=9)	0.5958 \pm 0.1390 (n=9)
14	0.5814 \pm 0.0483 (n=2)	0.5863 \pm 0.0544 (n=3)

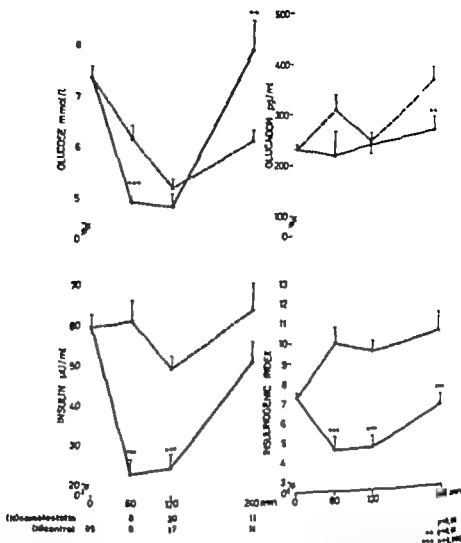


Fig. 4 Effect of a 40 min intravenous infusion of somatostatin (700 ng/kg/min) on glucose, insulin, glucagon and insulinogenic index in fed rats. The animals were killed by decapitation at 0, 60, 120 and 180 min (mean \pm S.E.). Filled circles and solid lines denote experiments with somatostatin, open circles and broken lines denote control experiments.

as on body weight and food consumption. Continuous infusion of somatostatin (700 ng/kg/min) for 96 h did not alter the levels of glycemia or of those of the above hormones or cyclic AMP in rats fed at libitum (Table 1). No glucosuria was observed at daily checking of the urine. The concentrations of insulin and glucagon in pancreatic extracts were almost identical in somatostatin treated and control rats after 4 days of infusion (Table 2). Likewise quantitative histological studies of the islets of Langerhans did not reveal any effects of 4–14 days infusion of somatostatin on the areas of D-cells (Table 3).

Continuous infusion of somatostatin over 4 days neither influenced body weight nor consumption of

food and water (Fig. 6). The decrease in food intake during the first day of the experiment was due to the withdrawal of food on the first day. Moreover somatostatin infused for 4 days did not influence body weight or food and water intake (Fig. 7). In these experiments somatostatin infusions were prepared every 4 h.

Plasma levels of somatostatin in rats continuously infused with somatostatin. Under basal conditions somatostatin levels in peripheral blood varied between 0 and 70 pg/ml. In animals infused with somatostatin (700 ng/kg/min) already 10 min after the start of infusion plasma levels of the hormone reached levels of approximately 500 pg/ml and remained almost constant for 4 h (Fig. 8).

4. synthesis and release of insulin from isolated pancreatic islets. *Diabetes & Metabolism* 2: 199-202.
5. SON B, HELLERSTRÖM C & HELLMAN U. 1972. Some characteristics of the two types of A cells in the islets of Langerhans of guinea-pigs. *Acta Endocrinologica* 57: 559-566.
6. H. WALIGORA, K., MATTHEWS J & STONE, B. 1977. Inhibition by somatostatin of glucose-induced glucose release from the isolated rat liver. *Endocrinology* 101: 1751-1759.
7. SMITH P H, WOODS, S C. & PORTE, D. 1976. Phenolamine blocks the somatostatin-mediated inhibition of insulin secretion. *Endocrinology* 98: 1073-1076.
8. WEEKS, J R. 1971. Manuscript January 1971. Available on request from J R Weeks Ph. D. Pharmacology Research The Upjohn Company Kalamazoo, Michigan 49001 USA.

explanation is supported by the observation that in anesthetized dogs the suppressive effect of somatostatin on insulin release could be counteracted by the alpha adrenergic blocker phenolamine (Smith et al 1976). In this context it is of interest that in perfused rat islets epinephrine inhibited glucose induced insulin release in a non competitive manner (Campfield 1975).

This work was supported by grants from Nordisk Insulin fond Gentofte Denmark Svenska Läkaresällskapet Forskningsfond Karolinska Institute and the Swedish Medical Research Council (13X-02397).

REFERENCES

- ARIMURA A, LUNDQVIST G, ROTHMAN J, CHANG R, ELDE R, COY D H, MEYERS C & SCHALLY A V 1978 Radioimmunoassay of somatostatin. *Metabolism* 7 1139-1144.
- BRAZEAU P, RIVIER J, VALE W & GUILLEMIN R 1974 Inhibition of growth hormone secretion in the rat by synthetic somatostatin. *Endocrinology* 94 184-187.
- BROWN M, RIVIER J & VALE W 1976 Biological activity of somatostatin and somatostatin analogs on inhibition of arginine-induced insulin and glucagon release in rats. *Endocrinology* 98 336-343.
- CAMPFIELD L A 1975 The effect of physiological concentrations of norepinephrine and epinephrine on the secretion of insulin. Thesis University of California Los Angeles.
- CHERRINGTON A D, CALDWELL H D, DIETZ M R, EXTON J H & CROFFORD O B 1977 The effect of somatostatin on glucose uptake and production by rat tissue in vitro. *Diabetes* 6 740-748.
- CHRISTENSEN B E, HANSEN Aa P, WEEKE J & LUNDBAEK K 1978 4-hour studies of the effect of somatostatin on the levels of plasma growth hormone, glucagon and glucose in normal subjects and juvenile diabetics. *Diabetes* 27 300-306.
- DUNBAR J C, WALSH M F & FOA P P 1978 The resumption of glucose response to glucagon: suppression with somatostatin, insulin or antihyperglycemic serum in depancreatized rats. *Diabetologia* 14 53-58.
- EDÉN S, ALBERTSSON WIKLAND K & ISAKSSON O 1978 Plasma level of growth hormone in female rats of different ages. *Acta Endocr* 88 676-690.
- EFENDIĆ S, GRILL V & LUFT R 1975 Inhibition by somatostatin of glucose induced 3, 5-monophosphate (cyclic AMP) accumulation and insulin release in isolated pancreatic islets of the rat. *FEBS Lett* 55 131-133.
- EFENDIĆ S, HÖKFELT T & LUFT R 1978a Somatostatin. In: *Advances in metabolic disorders* vol 9 pp 367-424. Academic Press, New York, San Francisco and London.
- EFENDIĆ S, LINS P E & LUFT R 1978 Somatostatin and insulin secretion. *Metabolism* 27 1175-1281.
- EFENDIĆ S & LUFT R 1975 Studies on the inhibitory effect of somatostatin on glucose induced insulin release in the isolated perfused rat pancreas. *Endocr* 78 510-515.
- EFENDIĆ S, NYLÉN A, ROOVETE A & NÄS-WALLENSTEN K 1978b Effect of arginine and arginine on the release of insulin and somatostatin from isolated perfused rat pancreas. *FEBS Lett* 97 33-35.
- FALOONA D E & UNGER R H 1974 Inhibition of hormone radioimmunoassay (ed B W A H R Behrman) p 324. Academic Press, New York and London.
- FELIG P & WAHREN J 1976 Somatostatin in diabetes. Suppression of glucose absorption than stimulation of glucose disposal. *Med* 25 1509-1511.
- GERICH J E, SCHULTZ T A, LEWIS S J, KARAM J H 1977 Clinical evaluation of somatostatin as a potential adjunct in the management of diabetes mellitus. *Diabetologia* 11 544.
- GILMAN A G 1970 A protein binding to adenosine 3, 5-cyclic monophosphate. *Proc Natl Acad Sci (Wash)* 67 305-312.
- GORDIN A, MEYERS C, ARIMURA A C, D H & SCHALLY A V 1977 Antihyperglycemic effect of arginine-induced insulin and glucagon release by somatostatin analogs. *Diabetologia* 8 833-841.
- GUILLEMIN R 1976 Somatostatin inhibits the effect of acetylcholine induced electrically in the vagus plexus. *Endocrinology* 99-1653-1654.
- HALES C N & RANDLE P J 1963 Immunoassay of insulin with insulin-antibody precipitate. *Biochem J* 88 137-146.
- HELLERSTRÖM C & HELLMAN B 1960 Studies of silver impregnation of the islets of Langerhans in the rat. *Acta Endocr* 35 518-532.
- HUGGET A S G & NIXON D A 1971 Glucose oxidase, peroxidase and 6-deoxyglucose termination of blood and urinary glucose. *Lab Invest* 2 368-370.
- JOHANSSON C, EFENDIĆ S, WISEN O I, NÄS-WALLENSTEN K & LUFT R 1978 Effect of short time somatostatin infusion on the propulsion in humans. *Scand J Gastroenterol* 13 423-424.
- KREIS G J, CONLON J M, DAVIS G B, KIN P, COLLINS S, MCCARTHY D M G, DON P & UNGER R H 1979 Somatostatin syndrome: clinical diagnosis of a patient and characterization studies in plasma and tumor tissue. *Endocrinology* 104 129.
- LOTTER E C & WOODS S 1977 Somatostatin decreases food intake. *Diabetes* 26 Suppl 1 158.
- OLIVER J N & WAGLE S R 1975 Studies on inhibition of insulin release, gluconeogenesis, gluconeogenesis by somatostatin in the rat. *Diabetologia* 11 777-777.
- OLSSON S E, ANDERSSON A, PETERSSON, HELLERSTRÖM C

ts of blood pressure manipulations on shivering
 nogenesis in the pigeon

ITOLA, S. SAARELA and R. HISSA

et of Zoology Zoophysiological Laboratory University of Oulu Finland

HOHTOLA, E. SAARELA, S. & HISSA, R. Effects of blood pressure manipulations on shivering thermogenesis in the pigeon. *Acta Physiol Scand* 1980, 110: 277-283. Received 5 February 1980. ISSN 0001-6772. Department of Zoology Zoophysiological Laboratory University of Oulu Finland.

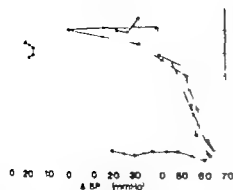
The effects of blood pressure (BP) manipulations on shivering thermogenesis were studied in conscious pigeons. A rise in BP induced by noradrenaline (NA) or equipotent doses of angiotensin II (Ang II) effectively suppressed shivering at +12°C and partly abolished the cold induced vasoconstriction in the foot. The inhibition commenced when the rise in BP reached +40 mm Hg, and fell as body temperature followed these responses. Comparison of the trajectories in the BP-shivering plane revealed that the inhibition of shivering by Ang II could be completely explained by changes in BP whereas NA had also another more prolonged inhibitory action independent of baroreceptor activity. A similar dose-dependence for effects on BP and shivering could be established with both drugs. An acute reduction of BP by sodium nitroprusside had also very potent inhibitory effect on shivering. Hypotensive pigeons elevation of BP with NA initially enhanced shivering, but when BP was raised beyond normal levels shivering was again suppressed. We conclude that both rise and fall in BP can inhibit shivering in the pigeon and that normal levels of BP facilitate shivering. NA inhibits shivering by more than one mechanism, but the initial effect is mediated through a baroreflexive action. The interactions of thermoregulatory and cardiovascular mechanisms suggest an integrated control of body temperature and circulation, which should be considered in experimental approaches to these homeostatic systems.

Key words: Blood pressure, shivering, baroreflexes, temperature regulation, noradrenaline, angiotensin II, sodium nitroprusside, pigeon.

erature regulation is usually defined as a osiatic system responding to changes in ther inputs reaching the controlling center. Several ury however, shows that a host of ological variables other than temperature af be effector functions of temperature regulation g Hammel 1968). These non-thermal inputs de cardiovascular variables, states of arousal, fernal factors, for example. They have some s been considered in models of body tem- ure control (e.g. Hammel 1968, Gale 1973), but nomic studies on the interaction of temperature dation with other homeostatic functions are few mber
 In this study we analyze the effects of blood ware (BP) manipulations on temperature reg- on in the pigeon. These experiments were nited by the observation that peripherally ad- minstrated noradrenaline (NA), known to stimulate

thermogenesis in new-born and cold-acclimated mammals (see Janaky 1973) suppresses cold-in- duced shivering in the pigeon (Hissa et al. 1975b) and other birds (Allen et al. 1970) resulting in de- creased heat production and lowered body tem- perature even in the cold-acclimated bird (Hissa et al. 1975). The profound cardiovascular effects of this drug and some earlier mammalian studies (viz. Mott 1963, Wasserstrum & Herd 1977) led us to suspect a baroreflexive origin for this inhibitory action. This contention was strengthened by the finding that the inhibition could be abolished with prior alpha-receptor blockade (Hissa et al. 1975b) which should reduce the pressor effects of peripheral NA.

Besides NA we used also angiotensin II (Ang II) more specific vasopressor agent, to induce elevations in BP. For lowering the BP sodium nit- roprusside (SNP) a potent vasodepressor acting



Traces of the changes in blood pressure (BP) were induced by injections of noradrenaline (solid line, closed circles) and angiotensin II (broken line, open circles). The arrows show the direction of time beginning at the moment of injection. The time interval between consecutive circles is 1 min.

amplifier was recorded without smoothing with a VEGG polygraph (Schwarzer).

Shivering was measured as the EMG-activity of the wing as measured in an EMG-processing apparatus consisting of an amplifier. The EMG was picked up by monopolar stainless steel electrodes fixed triangularly on the wing. The electrode length was 3 mm. The sides of triangle measured 3 mm. After insertion, the electrodes are connected to differential amplifier head-stage filter (the low and high cut-off frequencies 4 and 300 Hz, respectively). The relatively close arrangement of the electrodes attenuated cardiac potentials signal to some level and spurious potentials arising from respiratory movements (<1 Hz) are eliminated by head-stage filter. The amplified and filtered EMG-signal was full-wave rectified and averaged with an integrator (time constant 10 s). The averaged potential reflecting shivering intensity was recorded with the dual DP-6 potentiometer along with the mean arterial pressure. The direct EMG was continuously recorded with Tektronix 520A oscilloscope to ascertain true shivering potentials were obtained.

Body temperature (T_b) from the cloaca and the temperature of the naked tarsometatarsus (T_p) were recorded with small diode probes and digital thermometer consisting of our laboratory. Temperatures were plotted on a Clegg P250L-recorder.

Results

Seventeen pigeons were used. The experiments were begun by weighing the bird. 3 h after lights-on (6 a.m.) the bird was immobilized with hood (Evered & Fitzharris 1977) and tethered on the operating table in supine position with the wings extended. After exposing the cloacal vent, the BP-cannula and injection cannulae were inserted. Routinely NA was injected into the tibial vein and Ang II was given intramuscularly into the pectoral muscle. 5% NaCl was infused into the brachial vein. For injection the cannulae are connected to remote syringes.

The temperature probes and EMG-electrodes were fixed with adhesive tape and the bird was then returned to an upright position and placed into plethysmograph frame. The frame allowed the bird to attain normal postures of the wings head and body. The legs were tethered and bent towards the tail. The bird was then transferred into thermostatically controlled metabolic chamber maintained at $+17^\circ\text{C}$. This temperature elicited strong shivering in the restrained bird (peak-to-peak amplitudes of the EMG 800–1200 μV , mean rectified potential approximately 60 μV). The hood was removed, the chamber darkened and 0.5 h equilibration time was allowed before starting the measurements.

RESULTS

Fig. 1 shows the mean effects of BP elevations induced by NA (500 $\mu\text{g/kg}$ i.v., left panel) and Ang II (50 $\mu\text{g/kg}$ i.v., right panel). The rise in BP after NA-injection is followed by an inhibition of shivering, tachycardia, a fall in T_b , and a partial abolition of the cold-induced vasoconstriction in the feet. The rise in BP induced by an equipotent dose of Ang II likewise inhibited shivering, lowered T_b , and elicited foot vasodilatation. The response in HR was clearly biphasic.

After the responses had decayed, the level of BP settled 21 (± 9.8 S.E.) mmHg below preinjection level ($P < 0.05$, paired *t*-test) after NA, whereas after Ang II it settled 23 (± 3.9) mmHg above control level ($P < 0.002$, paired *t*-test). These shifts did not affect subsequent levels of shivering.

Although shivering was similarly suppressed by the elevation of BP with both drugs, marked difference was noted. While shivering returned to normal levels concomitantly with restoration of normal BP after Ang II injection, it was suppressed for a much longer time after NA, despite the re-

Table 1 Regression coefficients and their standard deviations relating the change in shivering to changes in blood pressure (BP) between $+40$ and $+65$ mmHg after injections of noradrenaline (NA) and angiotensin II (Ang II)

Phase of BP	Drug	
	NA	Ang II
Rising	-1.99 ± 0.28	-1.29 ± 0.11
Falling	-0.18 ± 0.05	-1.90 ± 0.30

The coefficient marked by an asterisk is significantly ($P < 0.001$) different from all other coefficients. Each coefficient is based on data from 6 pigeons.

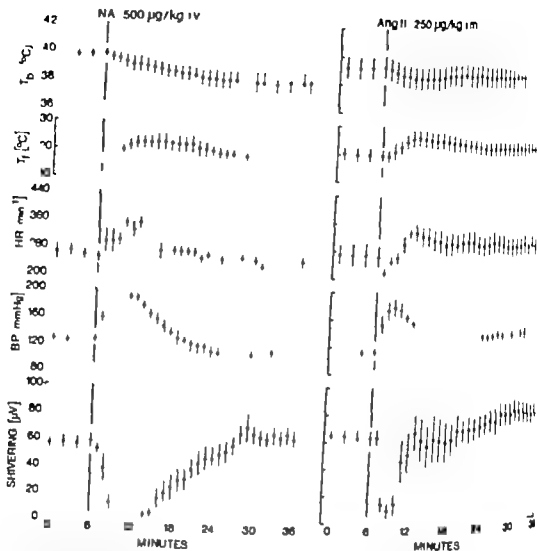


Fig. 1 Effects of noradrenaline (NA; left panel) and angiotensin II (Ang II; right panel) on shivering, blood pressure (BP), heart rate (HR), foot temperature (T_f) and body temperature (T_b) in the pigeon at an ambient temperature of $A = 6$ in both groups. The maximal changes are all significant ($P < 0.05$ or better, paired t -test).

directly on vascular smooth muscle (e.g. Kreye 1978) was used. By manipulating BP with these drugs, clear-cut and reproducible changes in shivering could be elicited. Preliminary results of these experiments have been presented elsewhere (Hohtola & Saarela 1979).

MATERIAL AND METHODS

Animals

Adult pigeons (*Columba livia*, mass 0.28–0.38 kg) of both sexes were used. They were housed in metal cages at $+22^\circ\text{C}$ in an animal room with a 12:12 L:D light-dark cycle and 40–50% RH for several weeks prior to experiment. Food and water were available ad libitum.

Drugs

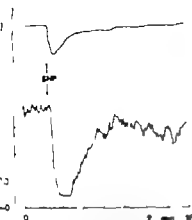
Noradrenaline (NA; L-arterenol bitartrate, Sigma Chemical Company) was dissolved in 0.85% NaCl. An-

giotensin II (Ang II; Sigma Chemical Company) was dissolved in buffered (pH 9) saline. Sodium azide (SNP; Sigma Chemical Company) was dissolved in saline. All solutions used for intravenous use were heparinized (approx. 100 U/ml). The concentrations were adjusted to give injection volumes of 1 ml.

Mean arterial pressure

Blood pressure (BP) was measured from the right brachial artery. A tapering polyethylene cannula (PE 50, F) with heparinized saline was introduced into the artery near the proximal end of the humerus and connected to a MSD10-S (Ailtech) pressure transducer. A 0.25 mm (Schwartz) strain gauge coupler was used to pull the transducer signal. The output of the coupler was fed into an RC-integrator (time-constant 10 s) and the mean pressures were thus obtained. It was recorded with a Radiometer DP-6 (Tohshin) potentiometer. All BP values in the present study refer to mean arterial pressure.

For measuring the effect of a parallel input



The effects of sodium nitroprusside (SNP 100 mg/kg) on blood pressure (BP) and shivering in a representative pigeon at 12°C

to be observed. Fig. 5 shows the responses of shivering to NA in a representative pigeon rendered hypotensive by controlled bleeding. The fall in BP is initially followed by an increase in shivering intensity, but the subsequent rise of BP to normal levels (i.e. about 120 mmHg) again suppresses shivering. In contrast to the effects of acute hypotension elicited by SNP (Fig. 4), shivering was initially inhibited by the chronically lowered BP.

DISCUSSION

Present findings show that BP can profoundly influence shivering in the conscious pigeon. Both a rise and a fall in BP result in a suppression of shivering. The discussion that follows is based on the evidence that baroreceptors have a major role in mediating these effects. However, it should be noted that baroreceptor activity was manipulated indirectly via BP-changes.

That the inhibition of shivering by peripherally administered catecholamines might be mediated indirectly through baroreflexive mechanisms has rarely been considered, and so far only in mammals. Wahren & Herd (1977) demonstrated a baroreflex-mediated depression of cold-induced oxygen consumption in the squirrel monkey, and while this error, as in preparation, Marshall & Stoner (1979) reported that shivering in the rat is inhibited when it is elevated by NA or methoxamine. That baroreceptor activity in general affects shivering

was suggested by the early studies of Ishii & Ishii (1960a, 1960b) and Mott (1963). The same authors noted especially that a reduction of BP has a potent inhibitory effect on shivering, which has been confirmed in the present study also for birds.

However, by comparing the effects of NA and Ang II it was possible for us to demonstrate that intravenous NA and possibly other adrenergic agents exert their inhibitory effect on shivering through two separate mechanisms: An initial baroreflexive suppression and another, more long-acting inhibitory mechanism (Fig. 2). Marshall & Stoner (1979) also mention that shivering did not always reappear in pace with restoration of normal BP after NA-injection in the rat, and Hall & Goldstone (1940) suggest that adrenaline inhibits shivering by more than one mechanism in the cat.

It is possible that the later effect is mediated by a direct action of NA on the CNS. In the pigeon, Hissa & Rautenberg (1974) showed that centrally applied NA inhibits shivering. In the pigeon and Hissa & Pyörälä (1977) were able to alleviate the effects of peripheral NA on shivering by prior intrahypothalamic injection of phentolamine (alpha-receptor blockade). However, chemoreceptor stimulation also inhibits shivering (Good & Sellers 1957; Mott 1963; Schafer & Wüstenberg 1976). Considering the finding that intravenous NA may elevate plasma pCO_2 in the pigeon (Hissa & George 1980) this mechanism cannot yet be excluded, nor can the possible direct effects on skeletal muscle be disregarded (Bowman & Nott 1969).

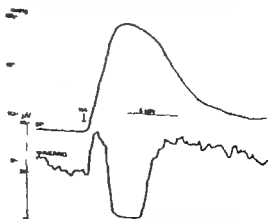


Fig. 5 Effect of NA (300 µg/kg) on blood pressure (BP) and shivering in a pigeon rendered hypotensive by controlled bleeding. Note the initial augmentation of shivering.

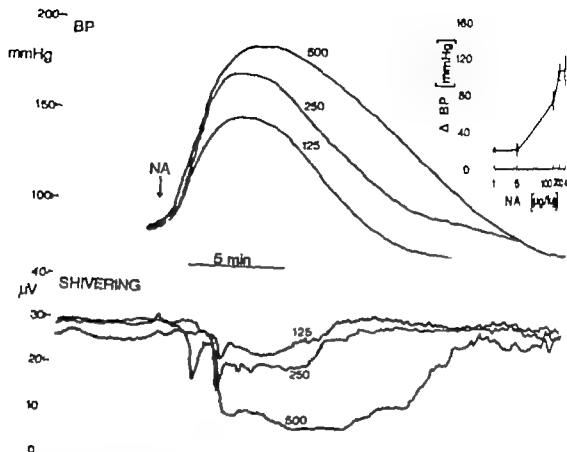


Fig. 3 Dose-dependent effects of noradrenaline (NA) on shivering and blood pressure (BP) in the pigeon. The curves shown along the curves are expressed as $\mu\text{g}/\text{kg}$. The inset shows the mean effects of NA on BP for a wider range of doses that was tested in preliminary experiments ($n=5$).

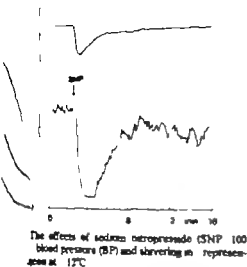
covery of normal BP. This correlated with the deeper fall in T_b after NA injection. The phenomenon is analyzed in more detail in Fig. 4 which shows the trajectories in the BP-shivering plane after injections of NA and Ang II. A clear-cut threshold effect is noted. Shivering begins to diminish when the increase in BP reaches about 40 mmHg and is completely suppressed after a rise of 65 mmHg. The superimposed curves for NA and Ang II coincide almost completely during the rising phase of BP. However, while the trajectory for Ang II follows an almost identical path also during the falling phase of BP, a marked hysteresis in the NA trajectory is observed at this phase, i.e. shivering remains suppressed despite the normalization of BP. In line with this, a linear regression analysis relating changes in shivering to changes in BP (between +40 and +65 mmHg) separately for the rising and falling phases of BP indicated that, excluding the falling phase of BP after NA-injection, the regression coefficients did not significantly differ from each other (Table 1).

The rise in BP needed to inhibit shivering in these

experiments was about 40 mmHg. The response showed however a dynamic nature for shivering was more abruptly elevated by injections into the brachial vein, for example shivering was immediately suppressed. In fact the injection rates were purposively selected to achieve such rates of increase in BP so that possible threshold effects could be evaluated.

By injecting NA at various dosages graded responses in BP were obtained. These were followed by graded inhibitions in shivering as well (Fig. 1). The rank order of potency of different NA doses was thus the same for changes in BP and shivering. Ang II also showed a similar dose-dependence when injected into the brachial vein, elicited on rises of BP at much lower dose rates (15–50 $\mu\text{g}/\text{kg}$). An acute reduction of blood pressure with 5% (100 $\mu\text{g}/\text{kg}$) had also a very potent inhibitory effect on shivering. The abrupt lowering of BP was immediately followed by cessation of shivering (Fig. 4).

When NA was injected into chronically hypertensive pigeons, an initial inhibition of shivering



As observed in Fig. 5 shows the responses of shivering to NA in a representative pigeon rendered hypotensive by controlled bleeding. The BP is initially followed by an increase in shivering intensity but the subsequent rise of BP to normal levels (i.e. about 120 mmHg) again inhibits shivering. In contrast to the effects of acute hypotension elicited by SNP (Fig. 4) shivering was only inhibited by the chronically lowered BP.

DISCUSSION

Present findings show that BP can profoundly influence shivering in the conscious pigeon. Both a rise and a fall in BP result in a suppression of shivering. The discussion that follows is based on the basis that baroreceptors have a major role in eliciting these effects. However, it should be noted that baroreceptor activity was manipulated indirectly via BP-changes. The inhibition of shivering by peripherally injected catecholamines might be mediated through baroreflexive mechanisms has rarely been considered and so far only in mammals. Warran & Herd (1977) demonstrated baroreflex depression of cold-induced oxygen consumption in the squirrel monkey and while this was in preparation, Marshall & Stoner (1979) noted that shivering in the rat is inhibited when it is elevated by NA or methoxamine. That receptor activity in general affects shivering

was suggested by the early studies of Ishii & Ishii (1960a, 1960b) and Mott (1963). The same authors noted especially that a reduction of BP has a potent inhibitory effect on shivering, which has been confirmed in the present study also for birds.

However, by comparing the effects of NA and Ang II it was possible for us to demonstrate that intravenous NA and possibly other adrenergic agents exert their inhibitory effect on shivering through two separate mechanisms. An initial baroreflexive suppression and another more long-acting inhibitory mechanism (Fig. 2). Marshall & Stoner (1979) also mention that shivering did not always reappear in pace with restoration of normal BP after NA-injection in the rat, and Hall & Goldstone (1940) suggest that adrenaline inhibits shivering by more than one mechanism in the cat.

It is possible that the later effect is mediated by a direct action of NA on the CNS in the pigeon. Hissa & Rautenberg (1974) showed that centrally applied NA inhibits shivering in the pigeon and Hissa & Pyörälä (1977) were able to alleviate the effects of peripheral NA on shivering by prior intrahypothalamic injection of phentolamine (alpha-receptor blockade). However, chemoreceptor stimulation also inhibits shivering (Good & Sellers 1957; Mott 1963; Schafer & Wünnenberg 1976). Considering the finding that intravenous NA may elevate plasma pCO_2 in the pigeon (Hissa & George 1980), this mechanism cannot yet be excluded nor can the possible direct effects on skeletal muscle be disregarded (Bowman & Nott 1969).

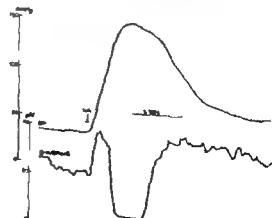


Fig. 5. Effect of NA (300 µg/kg) on blood pressure (BP) and shivering in a pigeon rendered hypotensive by controlled bleeding. Note the initial augmentation of shivering.

Two points strongly suggest that the effects of Ang II unlike those of NA are mediated solely by baroreflexes. The first is the lack of hysteresis in the BP shivering trajectory. Secondly, Görke & Pierau (1979) have shown that injection of Ang II (100 µg/kg i.v.) into spinalized pigeons has no effects on shivering. This experiment performed to alleviate the putative effects of low BP on muscle blood flow in spinal pigeons shows that the suppression of shivering by Ang II in intact pigeons is mediated by a mechanism involving CNS structures and not by direct effects on muscle blood flow.

The exact mechanism by which baroreceptor activity affects shivering is not clear. The pathways by which the rise and fall of BP mediate their effects are not necessarily the same. Besides the systemic effects of baroreceptor stimulation, a wide range of physiological functions including e.g. gamma motor activity (Schulte et al. 1959) is influenced by the baroreflex. The rapid onset of the responses suggests that a direct inhibition of efferent motor pathways controlling shivering is involved instead of systemic influences. Mott (1963) too concludes that the baroreflexive effects are mediated by inhibition of motoneurons. It is however known that a reduction of BP and the resultant decrease in baroreceptor activity induces an extremely pronounced vasoconstriction in skeletal muscle resistance vessels (see Kirchheim 1976). The ischemic effect of the vasoconstriction might have some influence on shivering in these experimental conditions.

Baroreflexes are often considered to be inert on skin blood flow (see Kirchheim 1976). However, the cold induced vasoconstriction in the feet of the pigeon was abolished with rising BP (Fig. 1), suggesting that baroreflexes affect skin blood flow in these conditions.

The experiment with hypotensive pigeons (Fig. 5) show that shivering can be enhanced by moderate baroreceptor stimulation. This is in line with the results of Ishii & Imai (1960a) and Mott (1963) who observed that shivering could be enhanced in the rabbit by moderate stimulation of the baroreceptor nerves or by NA and adrenaline injections after eliminating part of the afferent baroreceptor input by cutting the respective nerves. Furthermore, Ishii & Ishii (1960b) noted that the deviation from normal body temperature needed to elicit shivering was greater in rabbits that were hypotensive. These findings suggest that the normal

input to CNS from baroreceptors has a permissive or facilitatory effect of shivering.

Another point pertinent to the present is the integration of thermoregulatory and circulatory control mechanisms. Because both systems partly the same effectors, a physiological compromise is required when thermal and circulatory challenges are opposite. Morishima & Gale (1978) have demonstrated that thermal stimulation of the hypothalamus in the baboon can result in cardiovascular adjustments that are not affected by baroreflexes. This can be interpreted as a temporary suppression of baroreflexes by the thermoregulatory need of the organism (see also Coote et al. 1979). The present results and cited evidence from mammals show that a reciprocal suppression of thermoregulatory responses may also occur in conditions where changes in baroreceptor activity are pronounced. The interplay between these mechanisms is then not straightforward and it may depend on the magnitude of specific challenges presented. In any case, the reciprocal interactions suggest that thermal and circulatory responses are controlled in an integrated fashion. This conclusion is substantiated by the fact that the same supramedullary sites are involved in temperature regulation also partly in the integration of baroreflexes, at least in mammals (e.g. Hilton & Spyer 1971).

We conclude that both a rise and a fall in BP inhibit shivering thermogenesis in the pigeon. That NA inhibits shivering through a baroreceptor loop and also through another, possibly direct, route. These effects probably explain the hypothermic effects of NA found in birds at thermoneutral BP has a permissive effect on shivering. These interactions of thermoregulatory and circulatory control mechanisms should be considered in thermoregulatory experiments and procedures that affect the cardiovascular system.

This research was partly supported by Oulun yliopiston Tutkimuskeskus. We thank Hannu Rintamäki for helpful discussions.

REFERENCES

- ALLAN D. J., GARG R. N. & MARLEY E. I. (1969) Mode of action of α -methylnoradrenaline on temperature and oxygen consumption in young chicks. *Br. J. Pharmacol.* **24**, 667-687.
- BOWMAN W. C. & NOTT W. W. (1969) Adrenergic sympathomimetic amines and their antagonists. *Skeletal muscle Ph.* **2**, 21-27.

- J. H. 1978. Hypothalamic modulation of the receptor reflex. In: *Current studies of chemoreception* (ed. V. L. Veale & K. Lederis), p. 123. S. Karger, Basel.
- J. H. HILTON, S. M. & PEREZ-CONZALEZ, J. 1979. Inhibition of the baroreceptor reflex on stimulation in the brain stem defence centre. *J. Physiol. (Lond.)* 298: 549-560.
- J. D. M. D. & FITZIMONS, J. T. 1977. Techniques measuring isometrically induced drinking and of pressure changes in the conscious pigeon. *J. med. (Lond.)* 271: P4-5.
- C. C. 1973. Neuroendocrine aspects of their regulation. *Annu. Rev. Physiol.* 35: 391-430.
- A. L. & SELLERS, A. W. 1957. Effects of carbon dioxide, epinephrine and lidar on skin blood and rectal temperatures of unanesthetized dogs exposed to cold. *Am. J. Physiol.* 188: 451-455.
- E. K. & MIERAU, F. K. 1979. Inhibition of muscle activity in specialized pigeons during spinal cord cooling and warming. *Pflügers Arch.* 381: 47-57.
- V. E. & GOLDSTONE, P. B. 1940. The influence of epinephrine on shivering and on metabolism in the rat. *J. Pharmacol. Exp. Ther.* 68: 47-251.
- DEL, H. T. 1968. Regulation of internal body temperature. *Annu. Rev. Physiol.* 30: 641-710.
- J. N. S. M. & SPYER, K. M. 1971. Participation of anterior hypothalamus in the baroreceptor reflex. *J. med. (Lond.)* 218: 271-293.
- L. R. & GEORGE, J. C. 1980. Propranolol-induced metabolic changes and thermogenesis in the rat. *Comp. Biochem. Physiol.* 66C: 65-70.
- L. R. & PYÖRILÄ, A. 1977. Effect of α -hypothalamic phenolamine on hypothermia produced by peripheral noradrenaline in the pigeon. *Br. J. Pharmacol.* 61: 163-166.
- A. R. & RAUTENBERG, W. 1974. The influence of centrally applied noradrenaline on shivering and body temperature in the pigeon. *J. Physiol. (Lond.)* 238: 421-431.
- A. R. & PYÖRILÄ, A. & SAARELA, S. 1979. Effect of peripheral noradrenaline on thermoregulation in norepinephrine-acclimated pigeon. *Comp. Biochem. Physiol.* 51C: 43-47.
- HISSE, R. SAARELA, S. & PYÖRILÄ, A. 1975b. Thermoregulatory effect of peripheral injections of noradrenaline in the pigeon. *Comp. Biochem. Physiol.* 51C: 235-241.
- HOHTOLA, E. & SAARELA, S. 1979. Effects of blood pressure manipulations on shivering in the pigeon. *Acta Physiol. Scand., Suppl.* 473: 54.
- ISHII, K. & ISHII, K. 1960a. Carotid sinus reflex acting upon shivering. *Tohoku J. Exp. Med.* 72: 229-236.
- ISHII, K. & ISHII, K. 1960b. Relation of blood pressure to shivering. *Tohoku J. Exp. Med.* 72: 237-242.
- JANSKY, L. 1973. Nonshivering thermogenesis and its thermoregulatory significance. *Biol. Rev.* 48: 85-132.
- KIRCHHEIM, H. R. 1976. Systemic arterial baroreceptor reflexes. *Physiol. Rev.* 56: 100-176.
- KRAYE, V. A. W. 1978. Organic nitrates, sodium nitroprusside and vasodilatation. In: *Mechanism of vasodilatation* (ed. P. M. Vanhoutte & J. Leusen), p. 138. S. Karger, Basel.
- MARSHALL, H. W. & STONER, H. B. 1979. Catecholaminergic α -receptors and shivering in the rat. *J. Physiol. (Lond.)* 292: 7-34.
- MORISHIMA, M. S. & GALE, C. C. 1972. Relationship of blood pressure and heart rate to body temperature in baboons. *Am. J. Physiol.* 223: 287-295.
- MOTT, J. C. 1963. The effect of baroreceptor and chemoreceptor stimulation on shivering. *J. Physiol. (Lond.)* 166: 563-586.
- SCHAEFER, K. E. & WÜNNENBERG, W. 1976. Threshold temperatures for shivering in acute and chronic hypercapnia. *J. Appl. Physiol.* 41: 67-70.
- SCHULTE, P. J., HENATSCH, H. D. & BUSCH, G. 1959. Über den Einfluss der Carotissinus-Sensibilität auf die sympathomotorischen Systeme. *Pflügers Arch.* 269: 248-263.
- WASSERSTRUM, N. & HERD, J. A. 1977. Baroreflexive depression of oxygen consumption in the squirrel monkey at 10°C. *Am. J. Physiol.* 232: H451-H458.

Regional blood flow during reactive hyperemia in canine myocardium as determined by local washout of Xenon 133

HAUNSG and OLE AMTORP

Unit of Cardiology, Copenhagen County Hospital, Gentofte, Denmark

HAUNSG S & AMTORP O. Regional blood flow during reactive hyperemia in canine myocardium as determined by local washout of Xenon-133. *Acta Physiol Scand* 1980; 110: 283-293. Received 7 Feb. 1980. Department of Cardiology, Copenhagen County Hospital, Gentofte, Denmark.

The mechanisms regulating vascular tone in the myocardium were studied in open-chest anesthetized dogs by occlusions of the left anterior descending coronary artery (LAD) for 3 to 600 s. Cumulative excess blood flow (flow in excess of control flow) and repayment of flow debt (cumulative excess blood flow divided by blood flow deficit) were calculated using local injections of Xenon-133 for blood flow measurements. Release of vascular occlusion following 3 s of ischemia was not associated with any measurable hyperemia. Cumulative excess blood flow increased with increasing duration of ischemia from 5 to 600 s, but the increment in excess flow per unit extension of the occlusion time showed a considerable decline. Blood flow in excess exceeded blood flow debt occurred during the occlusion of 18 s duration of 161% with prolongation of ischemia to 600 s repayment of flow debt declined markedly to about 10%. Oxygen lack in the tissue elicited by perfusion of LAD—for 10 s with constant perfusion rate—with deoxygenated blood produced a fall in peripheral coronary resistance of about 40% which closely corresponds to the fall in resistance observed after period of LAD occlusion of similar duration. The results lead to the conclusion that vasodilator metabolites formed in the tissue during periods of arterial occlusion are of prime importance for the fall in the tone of the vascular smooth muscle cell occurring in the post-occlusion period. The findings argue against a myogenic component in this response.

Key words: Xenon washout, cumulative excess blood flow, autoregulation, peripheral coronary pressure, deoxygenated blood, vascular resistance.

the circulation in tissue is released after a fall of arterial blood flow is considerably lowered then returns to resting value after some time. This phenomenon of reactive hyperemia has been used in several experimental approaches to study factors determining vascular tone (Rusby 1926, Montgomerie et al. 1934, Abraham et al. 1941, Green & Weigle 1942, Folkow 1949, Brown 1956, Shepherd 1964, Lindberg 1966, Sørensen & Sejersten 1972, Henriksen et al. 1976). These studies have gained from quantification of the blood flow during hyperemia and repayment of flow debt incurred during periods of vascular occlusion. However, studies from myocardial tissue have been based upon measurement of absolute blood

flow recorded from a major coronary artery by the use of a rotameter or an electromagnetic flowmeter respectively. The results so far obtained suggest that the response of the vascular bed in the myocardium to acute arterial occlusion is due partly to myogenic partly to metabolic mechanisms.

The purpose of the present study was to re-investigate the characteristics of reactive hyperemia in the intact canine myocardium using a local Xenon-133 washout technique (Lassen 1967, Haunsg et al. 1979) which measures regional blood flow per gram of tissue. The effect of occlusion of left descending coronary artery (LAD) lasting from 3 to 600 s upon the reactive hyperemic response was studied. In other experiments the relaxation of the coronary resistance vessels in response to pure hypoxia was examined by measurement of peripheral coronary

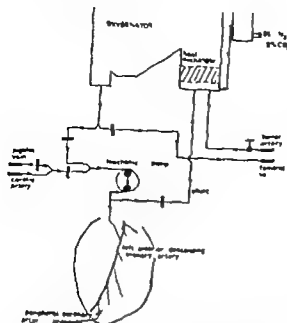


Fig. 1 The experimental preparation used to study the influence of oxygen lack upon the coronary vascular bed (for further explanation see text)

artery pressure during and following perfusion of LAD in 10 s with deoxygenated blood at a constant perfusion rate. The results indicate that vasodilator metabolites formed during myocardial ischemia are responsible for the post-ischemic hyperemia while myogenic mechanisms play little or no role.

METHODS

General experimental procedure

Studies were performed on 18 dogs weighing between 19 and 30 kg. Anesthesia was introduced by i.v. injection of thiomethural sodium (Leopental®) 1.5 mg per kg and respiration was maintained with oxygen and nitrous oxide through an endotracheal tube using a respirator. I.v. injections of fentanyl (Haldip®) 0.1 mg and pancuronibromide (Pavulon®) 2 mg were given when required.

A pigtail catheter was introduced through a femoral artery and the tip was placed in the ascending aorta. A Swan-Ganz flow directed thermodilution catheter (model 600855-22-6F) was inserted through the right external jugular vein with the tip placed in the pulmonary artery. Cardiac output was measured by injection of 5 ml 5% dextrose solution at 0°C into vena cava superior and calculated on a Devices (R3750) cardiac output computer. Through the left common carotid artery a catheter tip pressure transducer (Statham P-866) was placed in the left ventricle. The pressure signal was amplified and differentiated in a modified Elfab (4.4 BAIC) pressure monitor. The ventricular pressure P , its first derivative dP/dt and the aortic pressure wave form were recorded on an Elema Mingograph 34 III at a paper speed of 100 mm/s. Peak dP/dt was measured at the time just before opening of the aortic

valves. Aortic and pulmonary blood pressure were recorded continuously by means of a Statham P-23 Db) and together with ECG recorded on a U.V. recorder (model 4656) ABEM. Body temperature was maintained by external heating and recorded by a thermocouple thermometer placed in the rectum. The oxygen saturation, oxygen and carbon dioxide tension, pH and hematocrit were recurrently recorded. A thoracotomy was made and the pericardium was opened and sutured to the thoracic wall in order to minimize changes in the position of the heart due to movements. Atelectasis was minimized by continuous expiratory positive pressure.

Heart preparations. (a) The left anterior descending coronary artery (LAD) was isolated about 3 cm from origin and silk snare or a pneumatic occluder was placed around the artery to be used for occlusion. A small anterior branch of LAD was cannulated with a polyethylene heparin filled catheter (o.d. 0.5 mm) and the coronary artery pressure was measured by means of a Miller PC 150 tip catheter (o.d. 1.5 mm) with the tip inside the polyvinyl catheter. The ischemic area following coronary occlusion was about 15% of the entire myocardium. (b) LAD was isolated about 1 cm from its origin after the dog had been anaesthetized with heparin (400 U/kg). LAD was cannulated for perfusion with either arterial blood or blood with low oxygen concentration (cf. Fig. 1). Perfusion at a constant rate was achieved by a peristaltic rotary pump. During the experiment this was supported with blood from the carotid artery (pO_2 about 120 mmHg). By means of a cannula the blood supply to the pump could be switched to deoxygenated blood from an oxygenator (Pobasox term VT 7000) aerated with a gas mixture of 95% O_2 and 5% CO_2 . The oxygenator was fed with blood from the femoral artery. During LAD perfusion the carotid artery oxygenator blood was returned to a femoral vein. When switching to LAD perfusion with blood from the oxygenator blood from the carotid artery was returned into a jugular vein. The coronary perfusion pressure was approximately equalled systemic arterial blood pressure (within 10 mmHg). Peripheral coronary pressure was measured as described above.

Measurement of local blood flow

Myocardial blood flow in the region subjected to occlusion was determined before and following occlusion using the local Xenon-133 washout technique. Five μ l of Xenon-133 (10 mCi/ml or 40 mCi/ml) dissolved in sterile saline (obtained from the Radiochemical Centre, Amersham, England and AB Atomenergi, Stockholm, Sweden) was injected into that part of the myocardium of the left ventricle which could be subjected to occlusion. The depth of the local injection (5 mm) was secured by a collar on the needle.

The gamma radiation of Xenon 133 was measured with a scintillation detector with a thallium activated sodium iodide crystal placed 5 cm inside the opening of a lead collimator. The crystal was positioned at a distance of 14 cm above the anterior wall of the exposed left ventricle and collimated to obtain count rates from an area around the injection site.

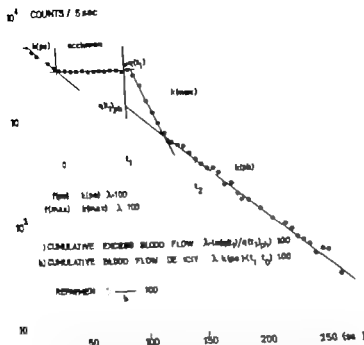


Fig 2 Representative washout curve following 60 s of vascular occlusion recorded after intramyocardial injection (depth 5 mm) of 5 μ l of Xenon-133. From the clearance curves washout rate constants before, during and after reactive hyperemia were obtained and excess cumulative blood flow and repayment was calculated as illustrated.

Denmark, RH 17 237) only gamma emission at 81 keV peak of Xenon-133 was recorded. The detector was connected to digital ratemeter and the counts were accumulated over periods of 1 or 5 s. The counting activity began immediately after each local injection of indicator.

Washout experiments are performed

In 18 dogs LAD was occluded for periods of 10 to 60 s at least 15 min interval between individual occlusions. In four of the dogs measurements were made using intramyocardial injection of 5 μ l of 133 Xe (90 mCi/ml) in order to achieve high contrast. With respect to background activity during the washout period. Results were similar to those obtained after intramyocardial injection of 5 μ l of Xenon-133 (90 mCi/ml).

In one dog measurements as described above were made after periods of vascular occlusion performed in random sequence (600 to 180 s).

In three dogs peripheral coronary pressure and blood flow were measured following occlusions of LAD lasting 4+8 (mean \pm S.D. = 9), 5.3 \pm 0.2 (mean \pm S.D. = 11), and 7.7 \pm 0.3 (mean \pm S.D. = 11), respectively.

In two dogs LAD was pump perfused with arterial blood at constant flow rate covering that peripheral coronary pressure equalled systemic arterial pressure. The perfusion of LAD was then abruptly switched to

perfusion—for 10 s with unchanged flow rate—with deoxygenated blood from the truncal aorta. Peripheral coronary artery pressure was continuously recorded.

() To assess the possible influence of blood clotting or platelet thrombosis upon the response to vascular occlusion, two additional dogs were heparinized with heparin sodium (400 U/kg) administered intravenously.

Aortic and pulmonary artery pressures, heart rate, cardiac output and peak dP/dt were measured before, during and following periods of ischemia. Experiments were discarded if these parameters during the washout period differed more than 10% from the pre-occlusive values. Changes in peak dP/dt of such magnitude were not observed in any of the occlusion experiments.

Calculations

Count rates of Xenon-133 corrected for background activity were plotted in semilogarithmic diagrams. A typical washout curve from a 60 s occlusion experiment is shown in Fig. 2. Assuming homogeneous perfusion, no arterial recirculation or veno-arterial shunting by diffusion and flow limited washout of the indicator the following parameters were calculated from the curves.

1. Pre-occlusive ($f(p_0)$) and post-hyperemic blood flow ($f(p_1)$) were calculated in accordance with the equation:

$$f(p) = \lambda \cdot 100 \text{ (ml min}^{-1} \text{ (100 g)}^{-1} \text{)}$$

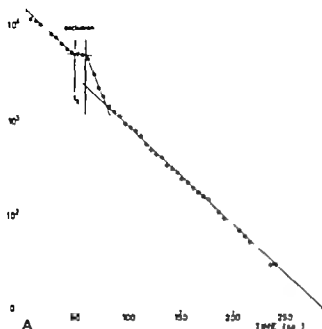
10^3 COUNTS/SEC

Fig. 3. Examples of Xenon-133 washout curves (A) 10 s and (B) 70 s of vascular occlusion. The parts of the curves have the same shape with similar slopes. About 5 s after vascular occlusion the curves are almost horizontal. After release, the prechemic disappearance rate of Xenon-133 decreases with a steep initial slope of the curves; the acceleration period of 5 to 10 s duration depends on duration of ischemia. The disappearance rate of ^{133}Xe returned to pre-occlusion values as determined the last part of the washout curves, both for monoexponential curves.

where k is the rate constant measured from the washout curve and λ is the tissue to blood partition coefficient in ml/g (Kety 1951). The k values in all experiments were obtained as slopes of the regression lines calculated according to the least square method using logarithmically transformed count rates corrected for background activity. The k values used were those given by Tomtesen & Sejrsen (1967) for skeletal muscle corrected from measured hematocrit values.

Blood flow values during reactive hyperemia were defined as follows:

Maximum blood flow (f_{\max}) was calculated from the k value corresponding to the initial steepest part of the Xenon-133 washout curve following release of arterial occlusion. In the experiment portrayed in Fig. 3, blood flow was stopped at $t = t_0$. The duration of the hyperemic period ($t - t_0$) was taken as the period from release of vascular occlusion ($t = t_0$) until blood flow had decreased to pre-occlusive level at time t .

Cumulative excess blood flow and repayment of flow debt per 100 g of tissue was calculated as described by Kristensen & Henniksen (1977) of equations in Fig. 3.

3. Cumulative excess blood flow V_{excess} which equals integrated blood flow during reactive hyperemia minus integrated pre-occlusive blood flow corresponding to the duration of the reactive hyperemia was calculated in accordance with the equation

$$V_{\text{excess}} = \lambda \ln(q(t_{\text{hyperemia}})) \cdot 100$$

where $q(t_0)$ is the activity at the time of release of vascular occlusion and $q(t)_{\text{pre}}$ is obtained by extrapolating the

activity versus time curve from $t = t_0$. (This also gives cumulative excess blood flow, whether the blood flow is constant or varies in the period t_0 to t .)

4. Repayment (R) of flow debt which equals cumulative excess blood flow divided by pre-occlusive blood flow. The times duration of ischemia was calculated in per cent accordance with the equation

$$R = \frac{V_{\text{excess}}}{f(t_0)} \cdot 100$$

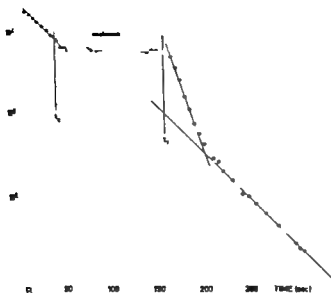
In the analysis of the results Student's t -test was used to determine the significance of differences between paired observations in each group and unpaired observations between different groups of experiments. A level of significance was chosen 0.05.

RESULTS

In Fig. 3A the artery was occluded for 10 s. Pre-occlusive blood flow was 97 ml/min/100 g. At release of the occlusion there was a short delay, thereafter a rapid increase in coronary blood flow. Thereafter blood flow decreased to the pre-occlusive value within about 70 s.

In Fig. 3B the artery was occluded for 70 s. Pre-occlusive blood flow was 104 ml/min/100 g. It appears that the washout of Xenon-133 delayed about 10 s at release of the artery.

COUNT/sec



ca. Thereafter the post-ischemic washout of 133 showed a rapid increase resulting in a low-slope. The disappearance rate of the isotope returned to the pre-occlusive value within 10 s. The washout rate after occlusion was determined from the last part of the washout which follows a monoexponential course. In all experiments the activity versus time curves became horizontal following vascular occlusion after a deceleration period (5–10 s) the initial rate of the occlusion. The washout rate after release of the vascular occlusion showed rapid increase which occurred after an acceleration period of 10 s duration depending upon duration of ischemia.

Results obtained in 69 experiments performed on 13 dogs (group +c) are summarized in Table 1. The initial rate constant obtained before vascular occlusion ($k_{(po)}$) and rate constants $k_{(pb)}$ obtained at the end of the post-hyperemic period did not differ significantly ($P > 0.05$). Maximum blood flow increased significantly when duration of ischemia was prolonged from 5 to 30 s ($P < 0.05$). Repayment of duration of ischemia: 60 120 240 600 s did not cause further increase in myocardial blood flow. The calculated cumulative flow of blood flow increased continuously with duration of vascular occlusion from 5 to 600 s.

Repayment of flow debt following 10 s of ischemia amounted to 260%. Following 30 s of ischemia repayment was reduced to around 100% and further marked reduction occurred with longer ischemic periods viz. to less than 10% following 600 s of ischemia.

No difference could be observed between the results obtained in 10 dogs (group a) and those obtained in one dog (group b) where the vascular occlusions of different duration were performed in the opposite sequence. Repayment during reactive hyperemia amounted in the latter to about 8% 47% 77% 130% and 299% following 600 120 60 30 and 10 s of ischemia.

In the experiments with brief occlusion periods (less than 10 s) and measurements of peripheral coronary pressure (group c) reactive hyperemia was not measurable following 3 s of occlusion. In all these experiments the coronary arterial pressure distally to the occlusion fell within 1 to 2 beats by 60 to 80 mmHg to a level of 10 to 20 mmHg, and rose rapidly to pre-occlusive values when the occlusion was released (Fig. 4).

In 10 experiments performed on dogs (group d) where LAD was perfused for 10 s with deoxygenated blood ($pO_2 = 16$ to 22 mmHg) peripheral coronary artery pressure fell from 103.4 ± 9.2 mmHg (means

Table 1 *Reactive hyperemia characteristics in 13 dogs obtained from increasing lengths of occlusion times*

In columns are shown (1) the length of occlusion time (2) the number of measurements corresponding to each occlusion period (3) the blood flow values measured from the washout curves of Xenon-133 before vascular occlusion (4) the flow values measured from the initial steep slope of the Xenon-133 washout curve during reactive hyperemia (5) duration of reactive hyperemia (6) blood flow values measured from the washout curves during the late post hyperemic period (7) the values of excess cumulative blood flow in the hyperemic period and (8) the values of λ_{excess} calculated from excess blood flow in per cent of blood flow debt. Figures are mean values \pm S.E.

Ischemic period (s)	Number of measurements (n)	Pre-occlusive blood flow $f(\text{po})$ ml (100 g) min	Maximum blood flow $f(\text{max})$ ml (100 g) min	Duration of reactive hyperemia (s)	Post-hyperemic blood flow $f(\text{ph})$ ml (100 g) min	Excess cumulative blood flow λ_{excess} ml (100 g)	λ_{excess} (%)
5	11	110.3 \pm 7.9	169.8 \pm 12.1	31.1 \pm 1.8	106.5 \pm 7.5	14.5 \pm 1.3	10.1 \pm 1.7
8	10	111.3 \pm 8.9	187.6 \pm 15.1	36.5 \pm 1.9	110.3 \pm 8.7	26.0 \pm 1.9	17.8 \pm 2.0
10	10	114.8 \pm 7.8	221.7 \pm 11.8	39.1 \pm 0	106.5 \pm 7.0	49.8 \pm 5.7	25.7
30	10	109.8 \pm 4.3	322.6 \pm 13.1	43.8 \pm 3.3	106.7 \pm 3.3	60.8 \pm 5.5	11.4 \pm 1.1
60	8	99.8 \pm 6.1	371.8 \pm 13.7	53.8 \pm 4.3	90.5 \pm 8.0	71.5 \pm 7.1	7.3
120	7	101.0 \pm 5.4	366.6 \pm 14.3	65.1 \pm 1.7	101.2 \pm 9.3	71.5 \pm 9.5	34.6
240	7	109.5 \pm 4.9	44.7 \pm 11.1	68.0 \pm 4.4	105.5 \pm 4.1	81.9 \pm 11.5	13.2
600	6	133.4 \pm 10.8	764.7 \pm 19.1	69.6 \pm 3.8	119.6 \pm 9.5	89.8 \pm 8.1	14.1

\pm S.D.) to 57.6 \pm 9.9 mmHg (means \pm S.D.) with a short delay of 3.8 \pm 0.5 s (means \pm S.D.) No changes in the rate of washout of Xenon-133 occurred in agreement with the fact that flow rate generated by the rotary pump was unchanged on switching from oxygenated to deoxygenated blood and back. Regional blood flow was calculated to 106 \pm 38 ml min (100 g) (means \pm S.D.) The fall in peripheral coronary vascular resistance amounted to 41%.

The results obtained with two heparinized dogs (group e) did not differ from those of unheparinized indicating that blood clotting and/or platelet thrombosis were not substantial factors in the hyperemic response.

DISCUSSION

Estimation of blood flow in control state and during reactive hyperemia from the washout of a freely diffusible indicator was used to study the mechanisms regulating vascular tone in the myocardium. The local washout technique of Xenon-133 used for calculation of regional blood flow and its application to myocardial tissue has been discussed in detail in a recent study (Haunsø et al 1979).

A crucial point in the calculation of blood flow is whether the tissue to blood partition coefficient of Xenon-133 (λ_{Xe}) changes during experiments due to tissue damage. The conclusion that λ_{Xe} does not change during the experiment is based upon the

observation that no difference could be detected between the calculated maximum post-occlusive blood flow values obtained when periods of ischemia were changed from 30 to 600 s or vice versa. Furthermore, blood flow values calculated during occlusive periods and late post-occlusive periods did not differ significantly indicating that myocardial tissue was not altered in composition to an appreciable extent.

The myocardial reactive hyperemic response noted in the present study was similar to that obtained by use of electromagnetic flowmeter. The local washout of Xenon-133 (residue method) shows initially after release of the vascular occlusion a short delay of 5 to 10 s during which the vascular bed in the counting field is filled with blood thereafter Xenon-133 activity vs time shows a fall representative of outflow of blood.

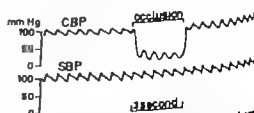


Fig 4 Effects of 3 s occlusion on peripheral coronary blood pressure (CBP) and systemic arterial blood pressure (SAP). Note that CBP is reduced by 60 to 80 mmHg during the occlusion and rises rapidly following the occlusion and rises rapidly to pre-ischemic blood pressure level when the occlusion is released.

100. The electromagnetic flowmeter (inflow) measures initially after release of vascular occlusion the inflow of blood into a partly empty vascular bed, and then registers blood flow in the tissue mass corresponding to the supply. The observed delay in the washout of ^{133}Xe corresponds to the electromagnetically measured delay (5 to 10 s) of attaining peak reactive blood flow rate.

101. The reactive blood flow of reactive hyperemia in the myocardium following periods of vascular occlusion is due to relaxation of vascular smooth muscle cells in coronary resistance vessels, i.e. arterioles and small arteries. The mechanisms underlying this relaxation have been discussed earlier. The myogenic and metabolic mechanisms have been discussed, but consensus has not been reached (Coffman & Gregg 1960; Coffman & Gregg 1961; Olsson et al. 1964; Olsson & Gregg 1965; Gellera et al. 1973; Eikens & Wilcken 1973, 1974; Bache et al. 1974; Olsson 1975; Giles & Wilcken 1977). According to the myogenic hypothesis, vascular smooth muscle cells will change their tone in response to changes in transmural pressure (Bayliss 1904; Folkow 1949; Patterson 1956; Sparks & Bohr 1964). This response is claimed to be initiated a few seconds and to be completed within 1 min. The metabolic hypothesis implies adjustment of blood flow to the metabolic demand of the myocardium, but the exact mechanism coupling blood flow and tissue metabolism is unsettled. It is well known that a number of vasodilator substances accumulate in the myocardium during occlusion: adenosine, bradykinin, carbon dioxide, histamines, l-histamine, lactic acid, potassium, uric acid and serotonin (Bernie 1964; Olsson 1975; Wayne et al. 1974; Rubio & Murray et al. 1979).

102. In the present experiments we have measured peripheral coronary artery pressure, estimated regional myocardial blood flow and calculated relative excess blood flow and repayment of debt incurred during periods of vascular occlusion. The occlusion reduced peripheral coronary pressure within 1 to 2 beats by 60 to 80 mmHg and the pressure returned immediately to occlusion level after release of vascular occlusion (Fig. 4). Maximum blood flow during reactive hyperemia increased with increasing length of vascular occlusion up to 30 s. Further prolongation of the duration of ischemia did not increase

maximum blood flow any further indicating that the resistance vessels in the coronary vascular bed were maximally dilated following 30 s of ischemia. This result is consistent with those reported by Coffman & Gregg (1960) and by Olsson (1975) but at variance with those obtained by other investigators (Olsson & Gregg 1965; Reneman & Spencer 1972; Bagger 1977; Dunn et al. 1979) who noted maximally vasodilatation to occur following 10 s of occlusion. It should be added that the present study did not include occlusions of between 10 and 30 s duration.

103. The cumulative excess blood flow in the myocardium following periods of occlusion of the supplying artery increased with increasing duration of the occlusion but the increment in excess blood flow per unit extension of the occlusion time declined considerably. These events may well be accounted for by accumulation of vasodilator metabolites in the tissue approaching an upper concentration with increasing duration of the occlusion time. During hyperemia these metabolites will be eliminated by washout, cellular uptake or/and metabolic conversion at a concentration dependent rate, e.g. first order kinetics.

104. Following brief occlusions of the coronary artery maximum blood flow and excess blood flow were considerably reduced and when occlusion time was shortened to 3 s, no increase in post-occlusion blood flow was detectable. The rate of change in perfusion pressure head was similar to that used by Sparks (1964) in human umbilical artery strips and might uncover a myogenic response of smooth muscle cells in coronary resistance vessels if existing. The results argue against a myogenic response playing an important role in post-occlusion hyperemia. The effect of metabolic factors on vascular tone was further studied by examining the influence of oxygen lack upon relaxation of coronary resistance vessels. Experiments were performed in which coronary artery was perfused for 10 s with deoxygenated blood at a constant flow rate. With an abrupt fall in arterial oxygen tension from about 120 to about 20 mmHg no changes in the rate of washout of ^{133}Xe occurred, but a fall in peripheral coronary pressure to about 41% was recorded within 4 s indicating a similar fall in vascular resistance. It is noteworthy that this decrement in vascular resistance closely corresponds to the observed decrement in vascular resistance following 10 s of vascular occlusion. In the short interval of

4 s from tissue perfusion with deoxygenated blood until a fall in peripheral coronary pressure was recorded metabolic disturbance in the myocardium was not sufficient to produce measurable vasodilatation. Correspondingly 3 s of vascular occlusion was apparently too short a period to induce oxygen lack resulting in significant release of vasodilator metabolites—while it should be sufficient to induce a myogenic response.

The high value for repayment following brief occlusions may be elicited by a fast rate of accumulation of vasodilator metabolites shortly after an occlusion—an accumulation that may be enhanced in the post-occlusion period by the transient increase in contractility force in the myocardium induced by ischemia of short duration (Theroux et al 1974). It is possible that vasodilator metabolites are formed at a reduced rate as myocardial contractions in the ischemic area cease when the arterial occlusion exceeds a certain duration. If it is so it may at least contribute to the marked fall in repayment observed with increasing duration of ischemia above some 60 s.

This study was supported by the Danish Heart Foundation and the Danish Medical Research Council. We wish to thank Dr Per Sejrsen and Dr Ole Henriksen for inspiring discussion and criticism of our study. We also wish to thank Mr s Dorte Friberg and Mr Morten Kjærungård for excellent technical assistance.

REFERENCES

- ABRAHAMSON D I, KATZENSTEIN E H & FERRIS E B 1941 Observations on reactive hyperemia in various portions of the extremities. *Amer Heart J* 22, 39–341.
- BACHE R, J COBB F R & GREENFIELD J C 1973 Effects of increased myocardial oxygen consumption on coronary reactive hyperemia in the awake dog. *Circulat Res* 33, 588–596.
- BACHE R, J COBB F R & GREENFIELD J C 1974 Limitation of coronary vascular response to ischemia in the awake dog. *Circulat Res* 35, 527–535.
- BAGGER H 1977 Regional blood flow in the left ventricular wall of dogs with a graded coronary artery stenosis. *Acta Physiol Scand* 100, 154–164.
- BAYLISS W M 1932 On the local reaction of the arterial wall to changes of internal pressure. *J Physiol* 23, 220–231.
- BERNE, R M 1964 Metabolic regulation of blood flow. *Circulat Res Suppl* 1, 14–15, 1261–1287.
- BERNE R M & RUBIO R 1974 Adenine nucleotide metabolism in the heart. *Circulat Res Suppl* III, 34–35, 109–118.
- COFFMAN J D & GREGO D E 1964 Reactivity characteristics of the myocardium. *Am J Physiol* 199 (6), 1143–1149.
- COFFMAN J D & GREGO D E 1964 Oxygen metabolism and oxygen debt repayment after occlusion of the coronary artery. *Am J Physiol* 201 (7), 841–847.
- DUNN R W, McDONOUGH, K. M. & GREGG, M. Jr 1979 High energy phosphate stores and levels in different layers of the canine left ventricle during reactive hyperemia. *Circulat Res* 41, 34–37.
- EIKENS E & WILCKEN D E L 1973 Myocardial reactive hyperemia and coronary vascular resistance in the dog. *Circulat Res* 33, 267–273.
- EIKENS E & WILCKEN D E L 1974 Ischemic hyperemia in the dog heart. Effects of increasing arterial inflow and of coronary occlusion lasting one and two cardiac cycles. *Circulat Res* 35, 702–711.
- FOLKOW H 1949 Intravascular pressure and regulating the tone of small vessels. *Acta Physiol Scand* 17, 289–310.
- FOLKOW H 1964 Description of the myogenic thesis. *Circulat Res Suppl* 1, 14–15, 1279–1281.
- GELLAL M, NORTON J H & DETAIL, I. Evidence for direct control of coronary vessels by oxygen. *Circulat Res* 32, 279–289.
- GILES R W & WILCKEN D E L 1977 Ischemic hyperemia in the dog heart: evidence for a myogenic contribution. *Cardiovasc Res* 11, 64–73.
- GOLDBLATT H 1926 Observations upon reactive hyperemia. *Heart* 1, 281–294.
- GREEN H D & WEGRIA R 1941 Effects of arterial anoxia and myocardial ischemia on the coronary flow. *Amer J Physiol* 135, 271–280.
- GUYTON A C, ROSS J M, CARRIER, O. J. & WALKER J R 1964 Evidence for local metabolic demand as the major factor causing myocardial reactive hyperemia. *Circulat Res Suppl* 1, 14–15, 140–149.
- HAUNSO S, AMTRUP O & LARSEN B 1971 Regional blood flow in canine myocardium is determined by local washout of a freely diffusible radioactive indicator. *Acta Physiol Scand* 106, 114–123.
- HENRIKSEN O, NIELSEN S L & PAASKE, S. 1976 Intrinsic regulation of blood flow in adipose tissue. *Acta Physiol Scand* 98, 30–36.
- KETY S S 1951 Theory and application of the change of inert gas at the lungs and tissues. *Pharmacol Rev* 3, 1–41.
- KRISTENSEN J K & HENRIKSEN O 1977 Evaluation of cumulative blood flow and metabolic repayment during reactive hyperemia. *Acta Physiol Scand* 109, 73A–74A.
- KRISTENSEN J K & HENRIKSEN O 1978 Cumulative blood flow and repayment during reactive hyperemia in human cutaneous tissue. *Acta Physiol Scand* In press.
- LARSEN N A 1967 On the theory of the local anesthetic method for measurement of blood flow and a discussion of its application to various tissues. *Med Scand Suppl* 472, 135–145.
- LINDBJERG I F 1966 Leg muscle blood flow measured with ^{133}Xe during periods of

- cular exercise performed during ischemia. *Clin Sci* 39:408.
- JOHNSON L. M., MOORE, J. M. & McQUINN, S. J. S. 1934 Reactive hyperemia. Relation of loss of increased blood flow to length of circulation. *Amer J Physiol* 108: 486-497.
- KAY P. A., BELLONI, F. L. & SPARKS, H. V. 1971 The role of potassium in the metabolic control of coronary vascular resistance of the dog. *Circulat Res* 25: 67-70.
- LENN, S. L. & SEJRSSEN P. 1972. Reactive hyperemia in subcutaneous adipose tissue in man. *Acta Physiol Scand* 85: 71-77.
- MACMILLAN, R. A. 1964 Kinetics of myocardial reactive hyperemia blood flow in the unanesthetized dog. *Circulat Res* 14: 1-15 81-85.
- MACMILLAN, R. A. 1975 Myocardial reactive hyperemia. *Circulat Res* 37: 263-270.
- MACMILLAN, R. A. & GREGG, D. E. 1965 Myocardial reactive hyperemia in unanesthetized dog. *Amer J Physiol* 201 (1): 224-230.
- OLSSON, G. C. 1946 The role of extravascular pressure in the causation of reactive hyperemia in the human forearm. *Clin Sci* 15: 17-25.
- OLSSON, G. C. & SPENCER, M. P. 1977 The use of reactive hyperemia to evaluate the coronary artery system. *Ann Thor Surg* 13: 477-487.
- OLSSON, G. C. & BERNE, R. M. 1975 Regulation of coronary blood flow. *Prog in Cardiovas Dis* 18: 105-121.
- SHEPHERD, J. T. 1964 Reactive hyperemia in human extremities. *Circulat Res*, Suppl. 1 14-15 76-79.
- SPARKS, H. V. 1964 Effect of quick stretch on isolated vascular smooth muscle. *Circulat Res*, Suppl. 1 14-15 1-254-1-260.
- SPARKS, H. V. & BOHR, D. F. 1962. Effect of stretch on passive tension and contractility of isolated smooth muscle. *Amer J Physiol* 202: 835-840.
- THEROUX, P., FRANKLIN, D. M., ROSS, J. & KEMPER, W. S. 1974 Regional myocardial function during acute coronary artery occlusion and its modification by pharmacologic agents in the dog. *Circulat Res* 35: 896-908.
- TJØNNESEN, K. H. & SEJRSSEN P. 1976. Inert gas diffusion method for measurement of blood flow. Comparison of bolus injection to directly measured blood flow in the isolated gastrocnemius muscle. *Circulat Res* 20: 552-564.
- WAYNE, A. R., KENT, K. M., PISANO, J. J., KEISER, H. R. & COOPER, T. 1975 Regulation of postocclusive hyperemia by endogenously synthesized prostaglandins in the dog heart. *J Clin Invest* 55: 1174-1181.
- WILLIAMSON, J. R., SCHAFER, S. W., FORD, C. & SAFER, B. 1976. Contribution of tissue acids to ischemic injury in the perfused rat heart. *Circulation* 53: Suppl. 1 1-3-1-14.

Taste and chewing as stimuli for the secretion of amylase from the parotid gland of the rabbit

GJÖRSTRUP

of Physiology and Biophysics, University of Lund, Sweden

GJÖRSTRUP P: Taste and chewing as stimuli for the secretion of amylase from the parotid gland of the rabbit. *Acta Physiol Scand* 1980, 110: 295-301. Received 13 Febr. 1980. ISSN 0001-6772. Institute of Physiology and Biophysics, University of Lund, S-223 62 Sweden.

Reflex secretion of amylase was studied in response to feeding pellets and carrots. Section of the ipsilateral glossopharyngeal nerve but not of the lingual nerve reduced the secretion of amylase with carrots but not with pellets. The fluid secretion was unaffected by the nerve sections. Larger volumes were secreted with pellets than with carrots, indicating that the hardness of the food, via chewing, is the main stimulus for fluid secretion. Sweet, salty, sour and bitter taste stimuli did not produce any fluid secretion in concentrations normally used for gustatory stimulation in rabbits. In a background flow of parasympathetically secreted saliva sweet stimuli regularly caused a large increase in the amylase output, salty stimuli usually had a small effect and sour and bitter stimuli seemed to have only exceptionally small effect. Citric acid in high concentrations caused fluid secretion, at the most at rate of about 50% of maximum. This saliva had a high content of amylase. The results support the view that during feeding the sympathetic secretory nerves can be activated via both taste stimulation and chewing, while the parasympathetic activity is mainly influenced by chewing.

Key words: Rabbit, parotid gland, amylase secretion, reflex secretion, taste, chewing.

Common knowledge that gustatory stimulation has secretion of saliva and the phenomenon has been extensively studied in both humans and animals, particularly in the parotid but to some extent also in the submandibular gland (e.g. Lashley 1916; Chacey & Shannon 1960; Kerr 1961; Smith & Kawamura 1967; Sobusha, Takashita & Ishii 1967; Kawamura & Yamamoto 1978). With respect to receptors (Baxter 1930, 1931; Newbrom 1962) investigations have dealt with the effects on salivary flow rate only and far less is known about the influence on the secretion of organic constituents. The information available suggests that taste is not necessarily linked to the rate of fluid secretion. Similarly, the flow rate has usually been the parameter used to evaluate the effects of chewing on salivary secretion (Lashley 1916; Kerr 1961). It is known that human parotid saliva obtained during conscious amylase (Newbrom 1962) secretion is of the kind of stimuli that lead to parasympathetic activation which is the main cause of the fluid secretion (see Ewing 1967) are better than low activation of oral receptors may

increase the sympathetic activity these nerves seem to have the principal control over the secretion of proteins, especially investigated as the secretion of amylase in rats (Schmeyer 1974; see Schramm & Selinger 1975; Garrett & Thalm 1975).

Experiments with electrical stimulation of the secretory nerves to the parotid gland of the rabbit indicate that in this gland a separate control of amylase and fluid secretion could be possible (Gjörstrup 1979, 1980). In conscious rabbits provided with parotid fistulae (Gjörstrup 1980) the feeding of carrots brought about a saliva with an amylase concentration 3-4 times as high as with pellets. In spite of this difference the two kinds of food stimulated to about the same high output of amylase, due to the much higher flow rates with pellets than with carrots. Thus the results indicated not only the existence of a separate activation of the sympathetic and parasympathetic nerves but also the possibility that more than one afferent nervous pathway may be involved in the control of amylase secretion, which has now been further investigated.

Taste and chewing as stimuli for the secretion of amylase from the parotid gland of the rabbit

GJÖRSTRUP

of Physiology and Biophysics, University of Lund, Sweden

GJÖRSTRUP P. Taste and chewing as stimuli for the secretion of amylase from the parotid gland of the rabbit. *Acta Physiol Scand* 1980; 110: 295-301. Received 13 Febr 1980. ISSN 0001-6772. Institute of Physiology and Biophysics, University of Lund, S-223 62, Sweden.

Reflex secretion of amylase was studied in response to feeding pellets and carrots. Section of the ipsilateral glossopharyngeal nerve but not of the lingual nerve reduced the secretion of amylase with carrots but not with pellets. The fluid secretion was unaffected by the nerve sections. Larger volumes were secreted with pellets than with carrots, indicating that the hardness of the food, via chewing, is the main stimulus for fluid secretion. Sweet, salty, sour and bitter test stimuli did not produce any fluid secretion in concentrations normally used for gustatory stimulation in rabbits. In a background flow of parasympathetically secreted saliva sweet stimuli regularly caused a large increase in the amylase output, salty stimuli usually had a small effect and sour and bitter stimuli seemed to have only exceptionally small effects. Citric acid in high concentration caused fluid secretion at the most at a rate of about 10% of maximum, this saliva had a high content of amylase. The results support the view that during feeding the sympathetic secretory nerves can be activated via both taste stimulation and chewing, while the parasympathetic activity is mainly influenced by chewing.

Key words: Rabbit, parotid gland, amylase secretion, reflex secretion, taste, chewing.

Common knowledge that gustatory stimulation increases secretion of saliva and the phenomenon has been extensively studied in both humans and animals, particularly in the parotid but to some extent also in the submandibular gland (e.g. Lashley 1930; Conway & Shauman 1960; Kerr 1961; Ueda & Kawamura 1967; Sahara, Takashita & Ueda 1967; Kawamura & Yamamoto 1978). With the exception of (Baxter 1910; 1931; Newbroom 1962) investigations have dealt with the effects on salivary flow rate only and far less is known about the influence on the secretion of organic components; the information available suggests that this is necessarily linked to the rate of fluid secretion. Similarly, the flow rate has usually been the parameter used to evaluate the effects of chewing on salivary secretion (Lashley 1936; Kerr 1961). It is known that human parotid saliva obtained during conscious amylase (Newbroom 1962) or the kind of stimuli that lead to parasympathetic activation, which is the main cause of the secretion (see Emanuel 1967) are better than low activation of oral receptors may

increase the sympathetic activity these nerves seem to have the principal control over the secretion of proteins, especially investigated as the secretion of amylase in rats (Schneyer 1974; see Schramm & Selinger 1975; Garrett & Thulin 1975).

Experiments with electrical stimulation of the secretory nerves to the parotid gland of the rabbit indicate that in this gland a separate control of amylase and fluid secretion could be possible (Gjörstrup 1979a; 1980). In conscious rabbits provided with parotid fistulae (Gjörstrup 1980) the feeding of carrots brought about saliva with an amylase concentration 3-4 times as high as with pellets. In spite of this difference the two kinds of food stimulated to about the same high output of amylase due to the much higher flow rates with pellets than with carrots. Thus, the results indicated not only the existence of a separate activation of the sympathetic and parasympathetic nerves but also the possibility that more than one afferent nervous pathway may be involved in the control of amylase secretion, which has now been further investigated.

METHODS

Surgical procedures

17 rabbits weighing ~2.8 kg were used. All surgery was done under pentobarbitone anaesthesia, 40 mg/kg given through a lateral ear vein, sometimes ether was added. To provide a salivary fistula the right parotid duct was exposed by an incision below the eye and cannulated with a polyethylene tubing (I.D. 0.38 mm \times O.D. 0.965 mm) that was bent subcutaneously backwards over the masseter muscle and led out through an incision behind the right ear (Gjørstrup 1979b). The patency of the system was tested by injections of methacholine 3–5 μ g/kg i.v. On the same occasion 10 of the animals had a second tubing of the same bore implanted with one tip entering into the mouth of the rabbit. By using the incision made for the cannulation of the salivary duct a wide bore injection needle was pushed from behind between the mandibular ramus and the masseter muscle in an antero-medial direction. Simultaneous intraoral palpation over the buccal region and the palate made it possible to guide the needle to penetrate the oral mucosa behind the last upper molar on the right side. The tubing was slipped through the needle leaving ~3 mm in the mouth and pointing forwards. The needle was removed and the tubing then led out behind the right ear beside the salivary cannula. 3–5 days later either the ipsilateral chordo-lingual nerve in 4 rabbits or the glossopharyngeal nerve in seven rabbits was cut. The chordo-lingual nerve was cut 5 mm laterally to the submaxillary duct and the glossopharyngeal nerve was sectioned near the exit from the skull where it branches. In two of the animals the section of the glossopharyngeal nerve was done a few days after the chordo-lingual nerve had been cut. In the other cases the two kinds of nerve sections were done in different animals.

Feeding experiments

The animals were fed either pellets or peeled carrots at intervals of 24 hours, on each occasion it was tried to feed the animal both kinds of food and from time to time to reverse the order in which it was given. During feeding the external end of the tubing coming from the salivary duct was arranged for saliva to drop into small test tubes, which were changed every 1 or 3 min. Each day 5–8 samples of saliva could usually be collected. The animals were calm during testing and did not show any signs of discomfort or impaired chewing due to the implanted tubings. A detailed description of this kind of experiment has been given elsewhere (Gjørstrup 1980).

Taste experiments

Through the polythene tubing implanted into the mouth various taste stimuli could be introduced without having to strain the animals which most often is the case when substances are to be given orally. The following substances (in reagent grade and dissolved in distilled water) were used as taste stimuli: Sucrose (0.5 M), sodium chloride (0.5 M), hydrochloric acid (0.02 M), quinine chloride (0.02 M), citric acid (0.5 M) and freshly pressed carrot juice. The taste solutions were injected at a speed of 5 ml/min which seemed sufficient for a good and continuous filling of the mouth since the animals usually

drooled slightly in spite of licking and swallowing. The solutions were generally given into the mouth freely with the exception of citric acid and carrot juice which were only given for 3 and 4 min at a time, respectively. During the infusion of the taste solutions saliva was usually collected in 1 or 2 min periods, the number of samples collected over 3 min. One min of rest was always allowed before sampling of saliva began. Taste solutions were given either when the parotid was quiescent or when it was secreting at a rate of 7–10 μ l/min. This fluid secretion was brought about by intragland injections of 0.3–0.4 ml of enzyme solution (1:10) into the gland through the tubing in the salivary duct (Emmelm et al. 1974). The secretion in response was allowed to build up for about 1 min before samples of saliva were collected during which time taste solutions were introduced. The periods of taste solutions were finished with rapid rinsing by sucking the mouth with ~10 ml of distilled water and then 1 min elapsed before control samples of saliva were collected.

Amylase and flow rate measurements

The samples of saliva were stored in deep frost and assayed according to the method described by Du (1964). In this method 1 unit of amylase is equivalent to the amount that liberates reducing groups corresponding to 1 μ mole maltose formed each minute at 37°C. Samples of saliva of 40 and 100 μ l were used and diluted 10–100 times before processing. The amylase secretion is expressed as the concentration of amylase in units/ml or the output of amylase in units/min. The observed values of saliva were measured with micropipettes, and the lowest measurement down to 10 μ l with an accuracy $\pm 1\%$.

Statistics

Statistical evaluation was performed with linear regression analysis and Student's *t*-test for unpaired observations. *P* values less than 0.05 were considered significant. Mean values are expressed as mean \pm S.E.

RESULTS

Figs 1a and 2a show, as has earlier been shown (Gjørstrup 1980) that the salivary flow evoked by the parotid gland when pellets are fed is 3–4 times larger than that obtained with carrots, but that in spite of this difference the gland is stimulated to produce an amylase secretion which for both kinds of food can reach the same high magnitude amount of 250 units/min. This amylase output remained unchanged with both pellets and carrots after section of the ipsilateral lingual nerve carried out in 4 rabbits (Figs 1b and 2b). Section of the ipsilateral glossopharyngeal nerve in 7 rabbits diminished the output of amylase on feeding carrots, as indicated by the significant reduction in the regression coefficient compared to that in the normal situation (*P* < 0.05).

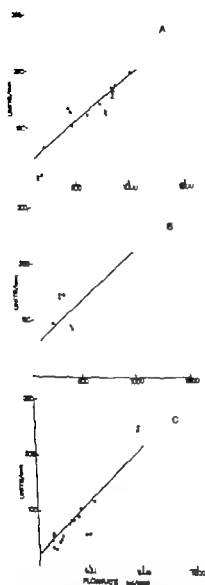


Fig. 1 Amylase secretion in response to pellets is shown as output versus the flowrate. The average secretion in each rabbit is shown as the linear regressions. (A) Normal innervation (regressor $Y=0.15 \pm 33.10$, $r=0.67$, $n=45$). (B) After section of the ipsilateral lingual nerve (regressor $Y=0.17 \pm 49.17$, $r=0.78$, $P<0.001$). (C) After section of the ipsilateral glossopharyngeal nerve (regressor $Y=0.19 \pm 20.30$, $r=0.72$, $P<0.001$). Observations from 9 rabbits.

2) while the output with pellets still could reach the magnitude obtained in rabbits with an intact innervation (Fig. 1). This reduction in output with carrots after section of the

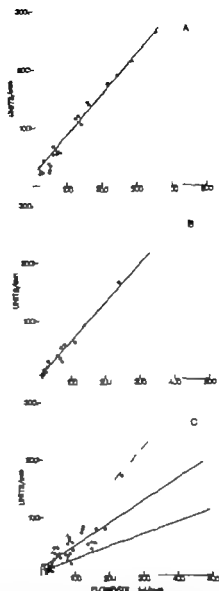


Fig. 2 Amylase secretion in response to carrots as the output versus the flowrate the same rabbits as in Fig. 1. (A) Normal innervation (regressor $Y=0.63x+14.79$, $r=0.88$, $P<0.001$, $n=49$). (B) After section of the lingual nerve (regressor $Y=0.65x+1.03$, $r=0.89$, $P<0.001$, $n=19$). (C) After section of the glossopharyngeal nerve (regressor $Y=0.39x+12.70$, $r=0.86$, $P<0.001$, $n=54$). In C the regressor under A (dotted line) and that obtained after preganglionic sympathectomy (broken line) from Göström (1980) have been inserted for comparison.

glossopharyngeal nerve varied from animal to animal. In some rabbits the reduction in amylase secretion was about 50% while in others it was less pronounced. After section of the lingual or the glo-

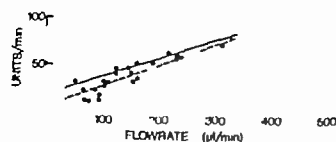


Fig. 3. Secretion of amylase in response to retrograde injections of exerine 0.3–0.4 ml of a solution with a concentration of 1 mg/ml. The output is shown versus the flowrate. To compare with the level obtained after preganglionic sympathectomy (broken line) from Gjørstrup (1980) linear regression analysis was performed $y = 0.19x + 19.79$ $r = 0.50$ $P < 0.01$ $n = 40$.

pharyngeal nerve flowrates as high as those observed in intact animals were still obtained, i.e. around 400 $\mu\text{l}/\text{min}$ with carrots and around 1000 $\mu\text{l}/\text{min}$ with pellets.

The salivary fluid secretion in response to taste stimulation with sucrose, sodium chloride, quinine chloride, hydrochloric acid and citric acid is summarized in Table 1. Apart from citric acid the power to provoke fluid secretion was poor; characteristically the salivary flow response to citric acid increased gradually and did not attain a high rate until a distinct behaviour of aversion was seen. The poor ability of the taste stimuli to cause fluid secretion made it necessary to provide a salivary flow when testing their effects on the amylase secretion. By retrograde injections of exerine into the glands, salivary flows within the range seen when feeding carrots were obtained (Fig. 3); the corresponding amylase output was low, being at the level recorded after preganglionic sympathectomy in a previous series of experiments (Gjørstrup 1980). When in this situation sucrose was administered into the mouth it caused a marked increase in the amylase content of the ongoing secretion, while the flow rate remained unchanged. On all occasions when sucrose was tested the amylase secretion was increased to at least the double compared to controls ($P < 0.001$) and most often much higher (Fig. 4a). At the continuous administration of sucrose into the mouth the amylase secretion regularly kept high for at least 7 min and then started to decline in some animals, but could in others keep up for the whole period of 5 min. The highest output of amylase in a single sample with sucrose was 343 units/min, collected at a flowrate of 180 $\mu\text{l}/\text{min}$. The amylase secretory effects in response to sodium chloride are sum-

marized in Fig. 4b. With this stimulus amylase secretion was more variable; in most cases a small increase was seen, about 10–20% of controls, and some sodium chloride failed to increase the amylase output and finally in two animals an increase about the double was recorded. When the mean of controls was compared to that of samples collected with sodium chloride the increase was found not to be significant. With sodium chloride the highest output of amylase was 159 units/min obtained at a flowrate of 715 $\mu\text{l}/\text{min}$. Hydrochloric acid and quinine chloride administered to 2 and 4 rabbits respectively did not cause any increase in the amylase output. In controls the output varied between 11 and 4 units/min, while in during test with hydrochloric acid and quinine chloride it was between 18 and 65 units/min, with the exception of one sample where quinine chloride was found to cause a release of 99 units/min. Carrot juice tested in 3 animals increased the amylase output by about 100% with the highest output at 154 units/min at 145 $\mu\text{l}/\text{min}$; these results are shown in Fig. 4c.

Citric acid, the only taste stimulus to evoke fluid secretion, also caused the release of amylase. Amylase secretion was measured on 4 occasions with an average of 683 ± 8 units/min and a corresponding output of 47 ± 30 units/min. The highest output obtained was 117 units/min at 450 $\mu\text{l}/\text{min}$.

DISCUSSION

The results clearly show that gustation is a powerful stimulus of amylase secretion in the rabbit. From the experiments with feeding carrots after section of the lingual or glossopharyngeal nerves on the ipsilateral side of the cannulated gland it may be inferred that the information is conveyed in the glossopharyngeal nerve. The unchanged level of

Table 1. Volume secretion responses to various gustatory stimuli injected introrotally continuously for 3–5 min at a rate of 5 ml/min

Substance (molar concentration)	Flowrate ($\mu\text{l}/\text{min}$)	Number of experiments
Sucrose (0.5)	<10	4
NaCl (0.5)	<10	6
HCl (0.01)	<10	3
Quininechloride (0.01)	<10	3
Citric acid (0.5)	763 \pm 5	7
Carrot juice (—)	<10	5

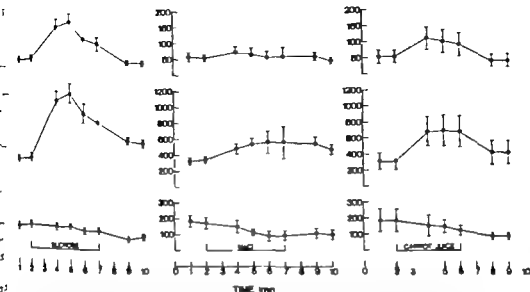


Fig. 1. Amylase secretion as output and as concentration in response to intranasal administration of solution of sucrose (0.5 M), sodium chloride (0.5 M) and carrot juice. The solutions were continuously injected at rate of 5 ml/min through cannula inserted polyethylene tubing emptying into the mouth. Since the test stimulus as themselves only evoked reflex secretion, they are administered in the presence of a fluid secretion obtained by injections of eserine into the mouth before zero-time. Samples of saliva were taken at one or two min intervals. Control samples were taken before and after administration of test stimuli. Saliva was not collected during the first min of administration of stimulus and not during the first min afterwards, due to deadspace and the time for the effects to reach steady state in or off at the end of the stimulation. Sucrose (each point mean \pm S.E. of 6-9 observations, Sodium chloride of 6-8 observations); Carrot juice (mean of 3 observations).

laryngeal secretion after section of the lingual nerve in accordance with experiments on decerebrate animals showing both secret secretion from the parotid gland on activation of the lingual nerve on the same side (Miller 1913). Further it is known that section of the lingual nerve makes the submaxillary but not parotid gland supersensitive to sialogogue drugs (Newman 1961). The reduction in amylase secretion after section of the ipsilateral glossopharyngeal nerve varied in extent from animal to animal and indicate an influence from the intact contralateral side that a unilateral input in the glossopharyngeal or lingual nerve can lead to activation of parotid or submaxillary glands on both sides has been demonstrated in experiments on several species (Miller 1913, Lashley 1916, Babkin 1928, Newman 1961). Sweet taste stimuli regularly caused a great increase in the amylase secretion while the effect of it was less powerful and occasionally even lacking. Bitter and sour as hydrochloric acid seemed to have no effect. A further example of the power of sucrose as stimulus for amylase secretion was that

it could evoke an output of amylase about as high as with isoprenaline injections, or as seen in normal animals reflexly secreting during feeding (Askew & Gjørstrup 1980, Gjørstrup 1980). An investigation in man indicates sucrose to be only slightly more effective than the other taste qualities in producing amylase secretion (Newman 1961) however for all stimuli the reported values are rather low. Carrot juice although subjectively less sweet than 0.5 M sucrose solution, caused a fairly high output of amylase as expected from the reflex secretion of amylase when feeding carrots (Gjørstrup 1979b, 1980).

The receptors innervated by the glossopharyngeal nerve of the rabbit have been shown to respond to all 4 basic tastes, using solutions of the same kind as in the present experiments (Appelberg 1958, Yamada 1967). The concentrations of the solutions used in the present investigation, except for citric acid and carrot juice have in whole nerve preparations of the chordo-lingual and the glossopharyngeal nerves of rabbits given about the same response measured as the integrated nervous activity (Beidler et al.

1955 Pfaffmann 1955 Yamada 1967) Recordings from single nerve fibre preparations in the chorda lingual nerve show all units to respond to the 4 basic tastes but at thresholds that for the single stimulus vary from one unit to another (Pfaffmann 1955 Beidler 1967). Thus it may be suggested that the prominent effect of sucrose on the amylase secretion is due to a specific pattern of discharge in the glossopharyngeal nerve that selectively activates the sympathetic nerves responsible for amylase secretion.

The high amylase output by pellets which do not have a sweet taste strongly suggests that other sensations or activities than gustatory can evoke secretion of amylase. An obvious explanation would be that chewing is the additional stimulus responsible for the activity in the sympathetic nerves in the rat chewing has been suggested as the main stimulus leading to amylase secretion (Schneyer 1974) and in man chewing of dental wax caused secretion of amylase but less than obtained by gustatory stimulation (Newbrun 1967). In the present experiments the assumption is further supported by the unchanged level of amylase output with pellets after section of the glossopharyngeal nerve. Chewing as additional stimulus for amylase secretion may also serve to explain why the output with carrots after glossopharyngectomy was not reduced to the level obtained after preganglionic sympathectomy.

Apart from citric acid the taste stimuli did not cause any secretion of fluid not even an increase in the flow caused by eserine. Hence it may be concluded that taste in this species is of less importance for the secretion of fluid than is the case in man or the dog (Lashley 1916 Baxter 1930 1931 Chauncey & Shannon 1960 Kerr 1961). The small fluid secretory effects are in agreement with experiments on both the submaxillary and the parotid glands of the rabbit (Kawamura & Yamamoto 1978 Suhara et al. 1967) compared to the volumes obtainable during feeding their recorded volumes in response to taste stimuli were very low. The greater fluid secretion effects with pellets than with carrots agree as earlier discussed (Gjørstrup 1980) with chewing as the main stimulus responsible for fluid secretion (Lashley 1916 Kerr 1961). Mechanical stimulation of the oral mucosa and particularly of the tongue is also known to cause secretion of saliva (see Babkin 1928) and the reflex was first electrophysiologically described in recordings from afferent and efferent

fibres in the chorda tympani of the rat (Hellebrandt & Kanahara 1973 a b) however in the rabbit the reflex seems to be of little importance during feeding since the flow of saliva was unchanged after section of the lingual or the glossopharyngeal nerves.

Citric acid may as stimulus for salivary secretion occupy a place apart from other taste stimuli, when given in high concentrations in order to elicit maximal secretory rates. In the rabbit a 0.5 M solution was followed by signs of aversion which in view of the poor responses to gustatory stimulation may be the main cause for the secretion. In the rat citric acid is shown to cause salivary flow rates at maximum (Emmett & Holmberg 1967), which is suggested to be the case also in man (Kerr 1961). In the parotid gland of the rabbit on the other hand citric acid could at the most produce a secretory rate at about half the maximum for the gland (Gjørstrup 1980) and in the submaxillary gland of the rabbit the secretion to noxious stimuli is reported to be even less (Kawamura & Yamamoto 1977 1978). The high amylase secretion indicates reflex activation of the sympathetic nerves in response to citric acid which has previously been demonstrated by Ohlin (1966) for fluid secretion in the submaxillary gland of the rat.

Supported by the Medical Faculty University of Lund Sweden

REFERENCES

- APPELBERG B 1958 Species differences in the taste qualities mediated through the glossopharyngeal nerve. *Acta Physiol Scand* 44: 129-137.
 ASHINO B & GJØRSTRUP P 1980 Amylase secretion in response to activation of different autonomic motor innervations in the rabbit parotid gland. *Acta Physiol Scand* 109: 407-414.
 BABKIN B P 1928 Die körpereigene Sekretion der Speicheldrüsen. ed. Springer, Berlin.
 BAXTER H 1930 The composition of the saliva in different phases of the secretion and by repeated stimulation. *Amer J Physiol* 91: 132-147.
 BAXTER H 1931 Further studies on the composition of saliva in different phases of the secretion. *Amer J Physiol* 97: 450-458.
 BEIDLER L M 1961 Dynamics of taste cells. In: *Olfaction and taste* (ed. Y. Zotterman), 1: 133-145. Pergamon, Oxford.
 BEIDLER L M, FISHERMAN I Y & HARDMAN W 1955 Species differences in taste responses. *Am J Physiol* 181: 235-239.

- LUNCEY III & SHANNON I. L. 1960 Parotid and secretion rate as method for measuring response gustatory stimuli in humans. *Proc Soc Exp Biol Med* 103 479-483.
- LQVIST A. 1961. A method for the determination amylase in salivary content. *Scand J Clin Lab Invest* 14 145-151.
- IELIN, N. 1961 Sensitization of the submaxillary gland by removal of part of the afferent flow to the salivary nuclei. *J Physiol* 157 402-409.
- IELIN, N. 1967 Nervous control of salivary glands. *Handbook of physiology: alimentary canal* (ed. C. C. Cade), Chapter 57 pp. 595-632.
- IELIN, N. & HOLMÖBERG J. 1967 Impulse frequency in secretory nerves of salivary glands. *J Physiol* 191 205-214.
- IELIN, N., MUREN A. & STRÖMBLAD R. 1954 Secretory and vascular effects of various drugs injected into the submaxillary duct. *Acta Physiol Scand* 7 225-238.
- IAKOSH, M. & KAWAMURA Y. 1967 Relations between taste qualities and parotid gland secretion in: *Olfaction and taste* (ed. T. Hayashi), 2: 281-300. Pergamon, Oxford.
- JEETI J. & THULIN, A. 1975 Changes in parotid secretory cells accompanying salivary secretion in rats on sympathetic or parasympathetic nerve stimulation. *Acta Res* 199 179-193.
- KSTRUP P. 1979 Amylase secretion in the rabbit parotid gland: local stimulating the sympathetic nerves and parasympathetic activity. *J Physiol* 296 443-451.
- KSTRUP P. 1979b A method for studying reflex secretion from the parotid gland of the conscious rabbit. *J Physiol* 296 299.
- KSTRUP P. 1980 Parotid secretion of fluid and amylase in rabbits during feeding. *J Physiol*, 309.
- LEKANT III & KASAHARA, Y. 1973 The lino-chloris tyrosine reflex — an electrophysiological microreflex. *Acta Physiol Scand* 87 199-207.
- LEKANT G. KASAHARA, Y. 1973b Secretory fibres in the lingual part of the lingual nerve to the sublingual salivary gland of the rat. *Acta Physiol Scand* 89 196-207.
- KAWAMURA, Y. & YAMAMOTO T. 1977 Salivary secretion to noxious stimulation of the trigeminal area. In: *Pain in the trigeminal region* (ed. D. J. Anderson & B. Matten), pp. 395-404. Elsevier/North Holland Biomedical, Amsterdam.
- KAWAMURA Y. & YAMAMOTO T. 1978. Studies on neural mechanisms of the gustatory-salivary reflex in rabbits. *J Physiol* 285 35-47.
- KERR, A. C. 1961 The physiological regulation of salivary secretions in man. *International series of monographs on Oral Biology* Pergamon Press, Oxford, London, New York, Paris.
- LASHLEY K. S. 1916. Reflex secretion of the human parotid gland. *J Exp Psychol* 1 461-493.
- MILLER F. R. 1913 On the reactions of the salivary centres. *Quart J Exp Physiol* 6, 57-72.
- NEWBRUN E. 1961. Observations on the amylase content and flow rate of human saliva following gustatory stimulation. *J Dent Res* 41 459-465.
- OHLIN P. 1968. Sympathetic secretory innervation of the rats submaxillary gland. *Quart J Exp Physiol* 53 19-22.
- PFÄFFMANN C. 1965 Gustatory nerve impulses in rat, cat and rabbit. *J Neurophysiol* 18: 429-440.
- SCHNEIDER, C. A. 1974 Role of sympathetic pathway in secretory activity induced in rat parotid by feeding. *Proc Soc Exp Biol Med* 147 314-317.
- SCHRAMM III & SELINGER, Z. 1975 Neurotransmitters, receptors, second messengers and responses in parotid gland and pancreas. In: *Stimulus-secretion coupling in the gastrointestinal tract* (ed. R. M. Case and H. Goebel) pp. 49-64. MTP Press Ltd, London.
- SUHARA, R., TAKASHITA H. & YAZAKI, S. 1967 Study on the parotid salivary secretion due to taste stimuli in rabbits. In: *Olfaction and taste* (ed. T. Hayashi), 2: 337-342. Pergamon Oxford.
- YAMADA, K. 1967 The glossopharyngeal nerve response to taste and thermal stimuli in the rat, rabbit, and cat. In: *Olfaction and taste* (ed. T. Hayashi), 2: 459-464. Pergamon, Oxford.

gradation of TRF Immunoreactivity in rat hypothalamus trahypothalamic brain and pancreas

JOUVUSALO

ate of Physiology University of Oulu, Finland

KOIVUSALO F Degradation of TRF immunoreactivity in rat hypothalamus, extra-hypothalamic brain and pancreas. Acta Physiol Scand 1980, 110: 303-306. Received 15 Feb 1980. ISSN 0001-6772. Institute of Physiology University of Oulu, Finland

The endogenous TRF as measured in radioimmunoassays of chromatographically purified extracts of excised hypothalamus, extrahypothalamic brains and pancreas was shown to be stable in intact organs and homogenates. However synthetic TRF added to tissue homogenates was degraded. Sonication of the homogenates liberated endogenous TRF for degradation. Chromatographically purified tissue extracts of hypothalamus, extrahypothalamic brain and pancreas were degraded similarly by rat serum. These results suggest that the major portion of endogenous TRF occurs in protected forms in tissues and hence the physiologically effective amount of TRF must be very low when compared to its total content in tissues.

Key words TRF pancreas degradation

have previously shown that several neural
gastrointestinal tissues after methanol extrac-
t, chloroform washing and cation exchange
omatology contain immunoreactive TRF
mäläso et al 1977). Our findings on pancreatic
F are confirmed by others using a boiling
died for extraction, followed by gel filtration and
away (Morley et al 1977), and by us with sever-
dial layer and high pressure liquid chromato-
graphy methods (Leppäaho et al 1978). It is sup-
posed the metabolic regulation of brain peptides
olives peptidase degradation (Kuhl & Tauber
ML and some of the cleavage products appear to
biologically active (Prasad et al 1977). The pur-
se of this study was to obtain more information
not tissue TRF by various degradation tech-
niques and especially to investigate if the pancreatic
TF is degraded in the same way as brain TRF.

ATERIAL AND METHODS

ection and determination of TRF Tissue samples
are homogenized in methanol, evaporated, washed with
pneum chloroform, purified by cation exchange chro-
matography and the TRF was measured by specific

radioimmunoassay as described elsewhere (Ling et al
1976, Leppäaho et al 1978).

*Degradation of endogenous TRF in excised intact or
gave* Excised hypothalamus, extrahypothalamic brains and
pancreata from decapitated rats were allowed to stand at
room temperature (20°C), for 0, 10, 30 and 90 min before
methanol extraction and determination of TRF. The time
for decapitation, section and sample weighing was 1-3
min. In another experiment rats were anesthetized with
Nembutal (35 mg/kg) and the skull was opened from
above. With the animal's anesthetized tissue samples were
taken from the cortex and the thalamo-hypothalamic area
and treated in two ways. Some samples were placed in
liquid N within 10 min and subsequently extracted in
methanol. Secondly samples were taken as above but
kept at room temperature for 3 min before they were
immersed in N₂. TRF was determined as described.

*Degradation of exogenous and endogenous TRF in
tissue homogenates* 10 hypothalamus, extrahypothalamic
brains and pancreata were crushed and homogenized in
PBS (5 ml/g) in teflon-glass homogenizer. Each tissue
homogenate was divided into 2 equal parts and to one half
synthetic TRF was added. The homogenates were incubated
at 37°C. Samples were taken in cold methanol at 0,
5, 10 and 30 min and TRF determined as described after
chromatography.

Sonication of tissue homogenates Tissue homogenates
were produced as in the previous experiment. No TRF
was added. Each homogenate was divided into 4 equal
parts and sonicated (MSR, England, Settings: power low

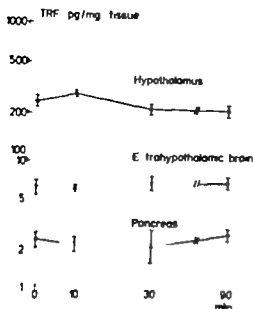


Fig. 1 TRF content in excised intact organs at -20°C as a function of time

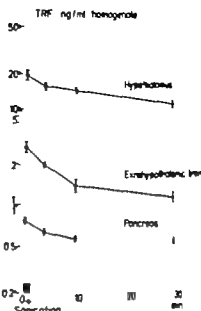


Fig. 3 Endogenous TRF-concentration in incubated (37°C) tissue homogenates after sonication

amplitude) for 1 min. The sonicated homogenates were incubated at 37°C and samples were taken at 0, 3, 9 and 27 min after sonication.

Degradation of synthetic TRF in rat serum. Synthetic TRF (0, 20, 200 and 2000 ng) was added to 1 ml of diluted rat serum (1:1 PBS) and incubated at 37°C . Aliquots were taken into cold methanol at various intervals for determination of TRF.

Degradation of purified tissue TRF by rat serum. Tissue homogenates were purified by cation exchange chromatography for TRF as described. The lyophilized

eluant was diluted in PBS (1:4 ml) and incubated with 1 μl serum at 37°C . Aliquots were taken as in the previous experiment.

All animals used were outbred Sprague-Dawley rats. They were handled in the normal way. All samples for one experiment were measured in the same assay. The values \pm S.E. ($n=4$) are given. If not otherwise indicated, the statistical significance was tested by Student's t -test.

RESULTS

When excised intact samples of hypothalamus, extrahypothalamic brain and pancreas were allowed to stand at room temperature for up to 90 min, there were no statistically significant losses of extractable TRF immunoreactivity (Fig. 1). In the experiment with rapid sampling from anesthetized animals, the thalamo-hypothalamic TRF concentration was 447 ± 85 pg/mg in samples frozen within 10 s and 404 ± 35 pg/mg in samples kept for 3 min at room temperature before processing. The respective values for cortical brain were 0.51 ± 0.20 and 0.69 ± 0.21 pg/mg. There were no statistically significant changes between values at 10 s and 3 min.

When synthetic TRF was added to tissue homogenates of hypothalamus, extrahypothalamic brain and pancreas, it was found to be degraded within 30 min, whilst the endogenous TRF in these homogenates was not degraded in this time (Fig. 2). Fig. 3 shows that when homogenates were sonicated for 1 min, there was a rapid decrease in TRF concentration during the first 10 min. This degra-

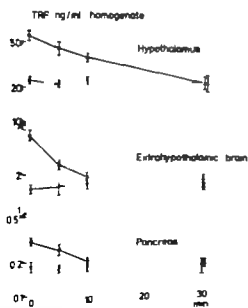


Fig. 2 Concentration of TRF in incubated (37°C) tissue homogenates without (●-●) and with (○-○) synthetic TRF added

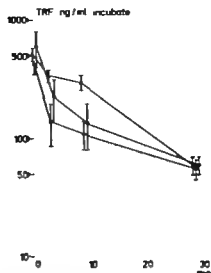
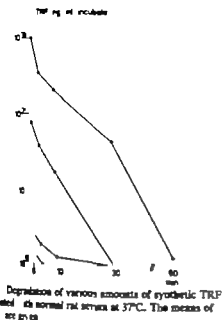


Fig. 5 Degradation of chromatographically purified tissue TRF when incubated with normal rat serum at 37°C. ●—● = hypothalamus, Δ—Δ = pancreas, ○—○ = extrahypothalamic brain.

of endogenous TRF in sonicated homogenates is similar in character to that of synthetic TRF in homogenates.

In the experiment where various amounts of synthetic TRF were added to normal rat serum the activity was destroyed (Fig. 4). The TRF activity from purified hypothalamic extrahypothalamic brain and pancreatic tissue extracts was reduced by normal rat serum to about 1/10 of the initial value in 30 min (Fig. 5).

DISCUSSION

Purify methanol extracts by cation exchange chromatography because we have found that simple ethanol extracts give higher TRF concentrations in RIA, especially if large amounts of tissue (10 mg) are used. This is not due to a loss of TRF in chromatography since the recovery of both synthetic TRF and H-TRF in our chromatography is 100%.

After the killing and excision procedures the tissues can be kept at room temperature with no loss of TRF immunoreactivity for up to 90 min. The experiment with rapid sampling gave no proof of a very rapid degradation immediately after death. If there are changes in the amount of TRF present

they must occur within 10 s of excision. These results show that, with recent excision techniques, degradation of endogenous TRF is not a problem.

The fact that exogenous but not endogenous TRF was rapidly destroyed in tissue homogenates indicates that endogenous TRF is well sheltered in the hypothalamus extrahypothalamic brain and pancreas all of which possess TRF degrading activity. Drastic manipulations such as sonication, alcohol extraction, sonic shock, etc., have to be used to liberate TRF from its protected form. Parker et al. (1978) have demonstrated that most TRF in the hypothalamus is located in the soluble fraction of the synaptosomes and is easily obtained from osmotically lysed synaptosomes. The subcellular locus of TRF in the pancreas is not known. It is also possible that TRF does not occur as a tripeptide in the tissue.

It is well known that synthetic TRF is rapidly degraded by serum and various tissues. Similarly normal rat serum degraded the TRF immunoreactivity from purified hypothalamic extrahypothalamic brain and pancreatic tissue extracts.

These degradation studies offer further evidence that the TRFs from pancreas and extrahypothalamic brain are identical with hypothalamic TRF and that in these tissues the TRF is protected in a similar way from an effective degrading

system Schaeffer et al (1977) showed that the release of TRF from hypothalamic synaptosomes is stimulated by K^+ and dependent on Ca^{2+} and that release can only be observed in the presence of a peptidase inhibitor. It is possible that the physiologically active TRF which is rapidly degraded is only a small part of the total TRF. Thus changes in TRF levels after stimuli to living animals are difficult to recognize. We have elsewhere reported a rapid rise in hypothalamic TRF content after stressful stimuli to rats (Koivusalo & Leppäluoto 1979) which is best explained as being due to the inhibition of the TRF degrading system. This together with the present findings indicates that the TRF degrading system is an important regulating mechanism in living animals.

The present study was supported by grants from Finska Läkaresällskapet.

REFERENCES

KOIVUSALO F & LEPPÄLUOTO J 1979 Brain TRF immunoreactivity during various physiological and stress conditions in the rat. *Neuroendocrinology* 29: 231–236.

KUHL, H & TAUBERT H D. 1975 Short loop feedback mechanisms of luteinizing hormone (LH)-releasing hypothalamic L-cysteine arginamide (LHRF) in the rat hypothalamus. *Acta Endo* 78: 649–663.

LEPPÄLUOTO J, KOIVUSALO F & KRAIVIL 1977 Existence of TRF-like immunoreactive in neuroectodermal tissues. *Proc Intern Union Phys Sci* 13: 440.

LEPPÄLUOTO J, KOIVUSALO F & KRAIVIL 1978. Thyrotropin-releasing factor: Distribution in neural and gastrointestinal tissues. *Acta Physiol Scand* 104: 175–179.

LING N, LEPPÄLUOTO J & VALE W M. Chemical, biological and immunological characterization of mono- and diiodothyrotropin releasing factor. *Anal Biochem* 76: 125–133.

MORLEY J E, GARVIN T J, PEARLY A E & HERSHMAN J M. 1977 Thyrotropin-releasing hormone in the gastrointestinal tract. *Br J Biophys Res Commun* 79: 314–318.

PARKER C R, NEAVES W B, BARNER J & PORTER J C. 1978. Studies on the subnuclear localization of luteinizing hormone-releasing hormone and thyrotropin-releasing hormone in the hypothalamus. *Endocrinology* 102: 1167–1173.

PRASAD C, MATSUI T & PETERKOFKY 1977. Antagonism of ethanol narcosis by levodopa line dioxetopiperazine. *Nature* 268: 144–144.

SCHAEFFER J M, AXELROD J & BROWNSTEN M J. 1977 Regional differences in dopamine-mediated release of TRF-like material from synaptosomes. *Brain Research* 138: 571–574.

Ins in relation to kallikrein activity, kininogen, electrolytes, aldosterone and catecholamines in urine from normal individuals

HULTHÉN J. F. DYMLING and B. HÖKFELT

Department of Endocrinology, Lund University Clinics, General Hospital S-201 40 Malmö, Sweden

HULTHÉN U. L. DYMLING J. F. & HÖKFELT B. Kinins in relation to kallikrein activity, kininogen, electrolytes, aldosterone and catecholamines in urine from normal individuals. *Acta Physiol Scand* 1980, 110: 307-314. Received 26 Feb. 1980. ISSN 0001-6772. Department of Endocrinology, Lund University Clinics, General Hospital, Malmö, Sweden.

The object of the present study was to test the hypothesis that urinary kinin excretion is an indicator of intrarenal kinin formation and to investigate urinary excretion of kinins in relation to natriuresis, kaluresis, diuresis and urinary aldosterone and catecholamines in normal individuals on free salt and water intake. In freshly voided urine collected from 4 normal individuals kinin concentration was directly related to kallikrein activity. Kininogen concentration was very low and neither related to kallikrein activity nor to kinin concentration. The excretion rates of kinins and kininogen were unrelated to the time interval between micturitions. In 24 hour urine collections from 10 normal individuals the excretion of kinins was positively correlated to natriuresis, kaluresis and diuresis and also to dopamine but not to aldosterone, noradrenaline and adrenaline. Kinin excretion was inversely related to age and was lower in women than in men. On the basis of these results it is concluded that urinary kinin excretion reflects intrarenal kinin formation in normal ambulatory individuals and that urinary kinins are formed mainly in the interstitial and vascular space of the kidney. Furthermore, kinins and dopamine seem to play physiological roles in the renal handling of electrolytes and water.

Key words: Adrenaline, aldosterone, diuresis, dopamine, kaluresis, kallikrein, kininogen, kinins, natriuresis, noradrenaline.

are potent vasodilatory peptides (Fox et al. 1962) occurring in human plasma (Hamberger 1964) and urine (Abe 1965). They are derived from substrate proteins, kininogens, by the action of kallikreins and/or activated plasma kallikrein (Nebeker & Pierce 1963). Injection of kinins into the renal artery increases renal blood flow and increases urinary excretion of sodium, potassium and water (Nebeker & Gilmore 1964). In the rat the effects of kinins and diuresis following an i.v. saline load are reduced by i.v. administration of bradykininase (Marm-Gruet 1974) or kallikrein inhibitor (Kraemer-Mack & Sieberer 1978). Renal and endocrine glands have been found in the kidney and in urine (Nustad, Vaae & Pierce 1971) and it has been assumed that urinary kallikrein excretion generally reflects renal kallikrein release and intrarenal kinin generation (Mills et al. 1976, Carretero & Scicli 1976). There is tentative

evidence that urinary kinin excretion is directly related to intrarenal kinin formation (Abe 1965).

To test the hypothesis that urinary kinin excretion is an indicator of intrarenal kinin formation, kinin concentration, kallikrein activity and kininogen concentration were measured in urine from normal individuals.

Furthermore the interrelations between urinary kinin excretion and natriuresis, kaluresis and diuresis were studied. As aldosterone, sympathetic activity and renal dopamine are implicated in renal handling of electrolytes and water (Gordon et al. 1965, Senley et al. 1972, Cuche et al. 1977, Alexander et al. 1974) urinary excretion of aldosterone and catecholamines were also determined.

MATERIAL

For the analysis of kallikrein activity, kininogen concentration and kinin concentration freshly voided urine

system. Schaeffer et al (1977) showed that the release of TRF from hypothalamic synaptosomes is stimulated by K^+ and dependent on Ca^{2+} and that release can only be observed in the presence of a peptidase inhibitor. It is possible that the physiologically active TRF which is rapidly degraded is only a small part of the total TRF. Thus changes in TRF levels after stimuli to living animals are difficult to recognize. We have elsewhere reported a rapid rise in hypothalamic TRF content after stressful stimuli to rats (Koivusalo & Leppäluoto 1979) which is best explained as being due to the inhibition of the TRF degrading system. This together with the present findings indicates that the TRF degrading system is an important regulating mechanism in living animals.

The present study was supported by grants from Finska Läkaresällskapet.

REFERENCES

- KOIVUSALO F & LEPPÄLUOTO J 1979 Brain TRF immunoreactivity during various physiological and stress conditions in the rat. *Neuroendocrinology* 29: 231–236.
- KUHL, H & TAUBERT H D. 1975 Short loop feedback mechanisms of luteinizing hormone releasing hypothalamic L-cysteine arginine releasing hormone (LHRF) in the rat hypothalamus. *Acta Endo* 78: 649–663.
- LEPPÄLUOTO J, KOIVUSALO F & KRAVITZ J 1977 Existence of TRF-like immunoreaction in neuroectodermal tissues. *Proc Intern Univ New Sci* 13: 440.
- LEPPÄLUOTO J, KOIVUSALO F & KRAVITZ J 1978 Thyrotropin-releasing factor: Distribution in neural and gastrointestinal tissues. *Acta Physiol Sci* 104: 175–179.
- LING N, LEPPÄLUOTO J & VALE W M 1978 Chemical, biological and neurotoxicological characterization of mono- and diiodothyrotropin releasing factor. *Anal Biochem* 76: 125–133.
- MORLEY J E, GARVIN T J, PEKARY A S & HFRSHMAN J M 1977 Thyrotropin-releasing hormone in the gastrointestinal tract. *Brain Biochem Res Commun* 79: 314–318.
- PARKER C R, NEAVES, W B, BARNES J & PORTER J C 1978 Studies on the subnuclear localization of luteinizing hormone-releasing hormone and thyrotropin-releasing hormone in the hypothalamus. *Endocrinology* 102: 1167–1174.
- PRASAD C, MATSUI T & PETERKOFKY I 1977 Antagonism of ethanol narcosis by levallorphan dihydrochloride. *Nature* 268: 142–144.
- SCHAEFFER J M, AXELROD, J & BROWNSTEN M J 1977 Regional differences in dopamine-mediated release of TRF-like material from synaptosomes. *Brain Research* 138: 471–474.

ns in relation to kallikrein activity kininogen electrolytes aldosterone and catecholamines rine from normal individuals

HULTHÉN J F DYMLING and B HÖKFELT

ment of Endocrinology Lund University Clinics, General Hospital S-21401 Malmö Sweden

HULTHÉN U L DYMLING J F & HÖKFELT B Kinins in relation to kallikrein activity kininogen electrolytes aldosterone and catecholamines in urine from normal individuals. *Acta Physiol Scand* 1980, 110: 307-314 Received 26 Feb 1980, ISSN 0001-6772. Department of Endocrinology Lund University Clinics General Hospital, Malmö Sweden.

The object of the present study was to test the hypothesis that urinary kinin excretion is an indicator of intrarenal kinin formation and to investigate urinary excretion of kinins in relation to natriuresis, kaliuresis, diuresis and urinary aldosterone and catecholamines in normal individuals on free salt and water intake. In freshly voided urine collected from 4 normal individuals kinin concentration was directly related to kallikrein activity. Kininogen concentration was very low and neither related to kallikrein activity nor to kinin concentration. The excretion rates of kinins and kininogen were unrelated to the time interval between micturitions. In 4 hour urine collections from 50 normal individuals the excretion of kinins was positively correlated to natriuresis, kaliuresis and diuresis and also to dopamine but not to aldosterone noradrenaline and adrenaline. Kinin excretion was inversely related to age and was lower in women than in men. On the basis of these results it is concluded that urinary kinin excretion reflects intrarenal kinin formation in normal ambulatory individuals and that urinary kinins are formed mainly in the interstitial and vascular space of the kidney. Furthermore, kinins and dopamine seem to play physiological roles in the renal handling of electrolytes and water.

Key words: Adrenaline aldosterone diuretics dopamine kaliuresis kallikrein kininogen, kinins, men, natriuretics, noradrenaline

are potent vasodilatory peptides (Fox et al. 1962) occurring in human (Hamborg 1965) and urine (Abe 1965). They are released from substrate proteins kininogens, by either kallikreins and/or activated plasma kallikrein (Webster & Pierce 1963). Injection of kinins into the renal artery increases renal blood flow and increases urinary excretion of sodium potassium water (Webster & Gilmore 1965). In the rat the diuresis and natriuresis following an intravenous saline load is reduced by intravenous administration of bradykinin (Marin-Grez 1974) or kallikrein inhibitor (Kraemer Moch & Slicher 1978). Renal glandular kallikreins have been found in glomeruli and in urine (Nisland, Vazir & Pierce 1975) and it has been assumed that urinary kallikrein excretion generally reflects renal kallikrein release and intrarenal kinin generation (Mills et al. 1976, Carrero & Scicli 1976). There is tentative

evidence that urinary kinin excretion is directly related to intrarenal kinin formation (Abe 1965).

To test the hypothesis that urinary kinin excretion is an indicator of intrarenal kinin formation, kinin concentration kallikrein activity and kininogen concentration were measured in urine from normal individuals.

Furthermore the interrelations between urinary kinin excretion and natriuresis kaliuresis and diuresis were studied. As aldosterone sympathetic activity and renal dopamine are implicated in renal handling of electrolytes and water (Gordon et al. 1965 Sealey et al. 1972, Cuche et al. 1972, Alexander et al. 1974) urinary excretion of aldosterone and catecholamines were also determined.

MATERIAL

For the analysis of kallikrein activity kininogen concentration and kinin concentration freshly voided urine

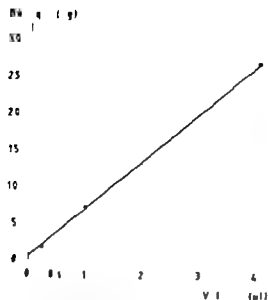


Fig. 1. Kinin formation (Bk eq) related to urinary volume. 0.1 ml human plasma substrate and 15 min incubation time was used.

was collected from 24 subjectively healthy individuals: 8 men and 16 women, aged 20–63 years. The time interval from the previous micturition was registered and urinary volume and pH recorded.

For the determination of urinary excretion of kinins: noradrenaline, adrenaline, dopamine, aldosterone, sodium, potassium, creatinine and water, 24-hour urine was collected from 50 subjectively healthy individuals: 1 men and 79 women, aged 23–77 years, in a plastic container with 10 ml concentrated glacial acetic acid.

Freshly voided urine was used in the first case because it was observed that acidification with glacial acetic acid gave an irreversible reduction of urinary kallikrein (kininogenolytic) activity. Acidification of the urine was used to preserve kinins (see below) and catecholamines.

METHODS

1. Kallikrein activity

Urine was stored under toluene at +4°C until processed in a single assay run. Urinary kallikrein activity was determined by incubating the urinary samples with human plasma substrate (HPS) and measuring the amount of bradykinin equivalents (Bk eq) formed. HPS was obtained from a normal individual by collecting blood in plastic tubes containing 0.2 mg hexadimethrine bromide (Polybrene, Aldrich-Europe, Belgium), 0.125 mg Soya Bean Trypsin Inhibitor (SBTI, Sigma, St. Louis, Mo) and 0.2 mg dipotassium-EDTA (Eastman Rochester, NY) per ml blood and separating the plasma by centrifugation. Polybrene was used to prevent plasma prekallikrein activation (Eisen 1964) and SBTI to inhibit activated plasma kallikrein (Werle & Maier 1952). Plasma collected in this way and stored at –20°C showed a stable kininogen level for months as measured by incubation with trypsin (TPCK 228 U/mg, 80% proteol, Worthington Biochem Corp, Freehold, NJ) or an excess of undiluted urine.

The incubation procedure was a modification of Cretter et al. (1976). Urine was diluted 1/100 in Tris-H buffer 0.1 mol/l, pH 8.3, containing dipotassium-EDTA 0.03 mol/l, 1,10-phenanthroline monohydrate (Fluka AG, Switzerland) 0.01 mol/l and 4,4'-bipyridine (Sigma) 0.001 mol/l. This buffer is referred to as TBEPH. 0.1 ml of diluted urine was incubated with HPS in 0.8 ml TBEPH for 15 min at +37°C. 120 µg of 1-lyl-3,4-His(4)-bradykinin triacetate (New England Nuclear, Boston, Mass.) was added as an internal standard. The enzymatic reaction was terminated by the addition of 1 ml ethanol 99.5% (v/v). Kinins formed were analysed by radioimmunoassay (RIA) as described for bradykinin in blood (Hult  n & Borge 1976). The antisera for bradykinin and lysyl-bradykinin (kallidin) equally reacted but did not cross-react with the major inactive peptide fragments of bradykinin. Recovery of (PH) bradykinin was 66 ± 7.1% (mean ± S.D.). 0.1 ml HPS incubated in TBEPH in the absence of urine corresponded to 0.4 Bk eq. This value was independent of incubation time and was subtracted from the values obtained in the test specimen.

When incubating 5 ng Bk with 0.1–0.5 ml HPS in TBEPH for 15 min, no degradation of Bk was observed. Increasing the urinary volume or the incubation time gave a linear increase in the amount of Bk eq formed (Fig. 1 and respectively). With volumes of HPS from 0.1 to 0.5 ml, maximal generation of Bk eq occurred with 0.1 ml (Fig. 3) and this was a consistent finding using HPS from several normal individuals. Adding 1.25 mg of SBTI instead of 0.125 mg per ml of blood for HPS did not decrease the amount of Bk eq formed with 0.1 ml HPS, but at less than 10% of the substrate was consumed as assessed by simultaneous incubation of HPS with trypsin and excess of undiluted urine (4.2 µg Bk eq/ml HPS formed). The intrasubject coefficient of variation (cv) for total kallikrein activity was 14.7%.

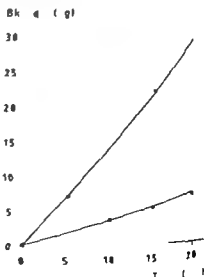


Fig. 2. Kinin formation (Bk eq) related to incubation times for two urinary samples. 0.1 ml human plasma substrate and 1 µl urine was used.

was directly prepared and incubated with trypsin in assay run according to Dinez & Carvalho (1963) (^3H) bradykinin was added to the incubate as an standard and the enzymatic reaction was terminated by the addition of ethanol 99.5% (1/1). Kinetics were measured by RIA (Häthén & Borge 1976). Recovery of (^3H) bradykinin was $74.5 \pm 7.8\%$. The assay cv for urinary kininogen was 11.1%.

Urine samples were frozen until assayed. Urine was pre-treated and determined by RIA as described by Häthén & Borge (1976). Recovery of (^3H) bradykinin, added as an standard, was $78.9 \pm 5.2\%$. Serial dilutions of urinary preparations gave lines, which were parallel to the (^3H) bradykinin standards in the RIA (Fig. 4). Generally volume corresponding to an original urinary volume of 1 ml was measured in each RIA tube.

Effect of acidification with glacial acetic acid (0.1 ml/ml urine) on urinary kinin concentration was studied in series of fresh samples from 4 individuals, which determined in single assay run. Kinin concentration was 36.6 ± 18.9 ng Bk eqv/ml (mean \pm S.D.) in non-acidified samples and 38.7 ± 18.5 ng Bk eqv/ml in acidified. The difference was not significant. The cv for the two samples from each individual was 11.0%. The correlation coefficient was 0.97 ($P < 0.001$).

Stability of kinins in acidified urine was studied in series of fresh samples from 24 individuals, which were determined in single assay run. Kinin concentration was 37.8 ± 19.1 ng Bk eqv/ml in samples frozen immediately after acidification and 39.1 ± 1.1 ng Bk eqv/ml in samples kept in room temperature for 4 h before they were assayed. The difference was not significant. The cv for the two samples from each individual was 9.5%. The correlation coefficient 0.97 ($P < 0.001$). The assay cv for urinary kinins was 12.0%.

Fig. 3 (ng)

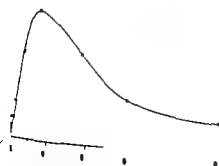


Fig. 3. Kinin formation (Bk eqv) related to volumes of plasma substrate (HPS). 1 μl urine and 15 min incubation time were used.

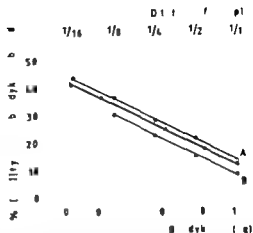


Fig. 4. Plot of percentage (^3H) tyrosine-bradykinin bound to antibody vs. log standard amount of bradykinin and log dilutions of prepared urinary samples respectively. Closed circles = standards of bradykinin. Open circles = dilutions of samples (A and B). Corresponding original urinary volumes were for A 80–5 μl and for B 20–5 μl .

IV. Noradrenaline, adrenaline and dopamine

Free catecholamines in urine were selectively isolated on cation-exchange resin column especially developed for this purpose by Bio-Rad Laboratories, Richmond, Cal., and eluted with boric acid (Roeth et al. 1971). Noradrenaline and adrenaline were measured spectrophotofluorometrically with the trihydroxyindole reaction (Euler & Floding 1965). Dopamine was quantified spectrophotofluorometrically after periodate oxidation (Anton & Seyre 1964; Roeth et al. 1971). The interassay cv for noradrenaline, adrenaline and dopamine was 10.7, 13.5 and 10.8% respectively.

V. Aldosterone

Aldosterone in urine was determined by RIA after acid hydrolysis and extraction on Sephadex LH 20 column (Pharmacia, Sweden) (Ito et al. 1972). Interassay cv was 13.2%.

VI. Urinary sodium, potassium and creatinine were measured by standard laboratory techniques. Urinary pH was determined with glass electrode.

STATISTICAL EVALUATION

Statistical analysis was performed by non-parametric methods. Mann-Whitney rank-sum test was used to detect differences between men and women, and Spearman rank-correlation to calculate correlation coefficients (r). The values are given as mean \pm S.D. and the level of significance is taken as $P < 0.05$.

Table 1 24 hour urinary excretion of the variables studied (Mean \pm S.D.)

	All subjects	Men (8)	Women (9)	Difference (8 vs 9)
n	50	1	79	
Age (y)	41 \pm 13	38 \pm 1	41 \pm 14	n.s.
Kinins (μ g Bk eqv/d)	4.3 \pm 15.3	48.5 \pm 15.6	37.5 \pm 13.3	P<0.01
Aldosterone (nmol/d)	5.6 \pm 1	77.3 \pm 13.4	24.3 \pm 11.1	n.s.
Dopamine (nmol/d)	1.624 \pm 551	1.884 \pm 605	1.436 \pm 427	P<0.01
Noradrenaline (nmol/d)	51 \pm 100	77 \pm 95	35 \pm 102	n.s.
Adrenaline (nmol/d)	98 \pm 5	73 \pm 7	48 \pm 16	P<0.01
Sodium (mmol/d)	147 \pm 60	168 \pm 64	111 \pm 53	P<0.02
Potassium (mmol/d)	6 \pm 1	70 \pm ...	55 \pm 2	P<0.02
Creatinine (mmol/d)	13.5 \pm 4.1	17.3 \pm 3.2	10.7 \pm 1.5	P<0.01
Volume (l)	1.31 \pm 0.41	1.53 \pm 0.31	1.15 \pm 0.41	P<0.01

RESULTS

In freshly voided urine kallikrein activity was 8.1 \pm 7.7 μ g Bk eqv/ml/15 min (incubation kininogen concentration 10.3 \pm 6.1 ng Bk eqv/ml and kinin concentration 78.3 \pm 13.1 ng Bk eqv/ml). Kinin concentration was positively correlated to kallikrein activity ($r=0.56$, $P<0.01$) but was neither related to kininogen concentration nor to pH (range 5.3–7.5). Kininogen concentration was not related to kallikrein activity but was negatively correlated to pH ($r=-0.67$, $P<0.01$). Neither kinin nor kininogen excretion (ng/min) was related to the time interval between micturitions (range 150–390 min).

In 24 hour urine the values for kinins, dopamine, adrenaline, sodium, potassium, creatinine and volume were higher in men than in women (Table 1). Kinin excretion was positively correlated to the excretion of dopamine, sodium, potassium and creatinine as well as to urinary volume but was not related to the excretion of aldosterone, noradrenaline and adrenaline (Table 2). There was an inverse

relationship between kinin excretion and age. The values for dopamine, noradrenaline and adrenaline were directly related to each other and showed positive correlations to the excretion of sodium and potassium as well as to urinary volume. Aldosterone excretion was directly related to noradrenaline excretion but did not correlate to renin or kalluretic and diuretic.

DISCUSSION

Most studies of the renal kallikrein-kinin system rest upon the determination of urinary kallikrein activity by esterolytic assays. The specificity of these assays has recently been questioned (Kopp, Mann & Bettels 1977; Levinsky et al 1979). Lack of specificity may explain some of the discrepant findings in earlier studies.

The level of urinary kallikrein activity found in the present kininogenolytic assay is somewhat higher than that reported by Carretero et al (1974).

Table 2. Interrelations between the variables studied (Spearman's rank correlation coefficients) (n=99)

	U-Kinins	U-Dopa- mine	U-Nor- adrena- line	U-Adrena- line	U-Aldo- sterone	U-Na	U-K	U-Crea- tinine	U-Vol- ume	Age
U-Kinins	x	0.45	0.20	0.19	0.77	0.48***	0.53	0.63	0.35	-0.37*
U-Dopamine		x	0.4	0.54	0.11	0.59*	0.52	0.62	0.35*	-0.18
U-Noradrenaline			x	0.30*	0.33	0.31	0.41	0.35	0.34	0.14
U-Adrenaline				x	0.2	0.35	0.41	0.46	0.40*	-0.21
U-Aldosterone					x	-0.06	0.27	0.3	-0.03	-0.13
U-Na						x	0.62*	0.50*	0.46	-0.25
U-K							x	0.55**	0.37*	-0.25
U-Creatinine								x	0.50*	0.25
U-Volume									x	0.27
Age										x

P<0.001 P<0.01 P<0.05

able explanation is that Carretero et al. (1976) partly purified kininogen from dog plasma as substrate. The decrease in Bk eqv formed when using the volume of HPS beyond 0.1 ml is in accordance with the finding of Martín-Grex & Carretero (1972) using heated dog plasma as substrate. It may be due to kallikrein inhibitory activity in plasma substrate (McConnell 1972).

Flow studies in the dog indicate that renal kinin is secreted into the urine in the distal part of nephron (Socli et al. 1976) and that kinins appear in the urine at this level (Socli, Gandolfi & Iero 1978). When injecting bradykinin into the urinary in dogs, urinary kinin excretion did not increase (Abe 1965). The present finding of a positive correlation between kallikrein activity and kinin concentration in fresh urine supports the concept that both these variables reflect the activity of renal kallikrein-kinin system in normal individuals on a free salt and water intake. Abe et al. (1978) also found a direct relation between urinary kallikrein and kinins in samples from normal individuals obtained before and following an intravenous injection of furosemide.

The origin of urinary kininogen has not been established. The concentration presently found is less than 1% of the kininogen level in human plasma (Abuja & Sepia 1971). Similar values were reported by Pisano et al. (1978). The lack of correlation between kininogen concentration and kallikrein activity and between kininogen excretion and kinin concentration indicates that the time interval between micturitions indicates that the low kininogen level is not primarily due to the loss of kallikrein in the urinary tract. The indirect relationship between kininogen concentration and urinary pH supports some pH-dependent formation of kinins in the urinary tract but kinin concentration does not correlate to urinary pH. Accordingly the main part of urinary kinins seems not to be formed in the urine.

Renal kallikrein is present in the interstitial and vascular space of the kidney (de Bono & Mills 1974; Carretero et al. 1976), where the conditions for kinin generation are more favourable than in the urine with respect to kininogen level (Werle & Zack 1970; Abuja & Sepia 1971) and pH (Görger, Maun & Eich 1977). Kinins formed in this location may enter the urine at the level of the distal tubule where the capacity for enzymatic cleavage of kinins is very limited (Carone et al. 1976).

The lack of correlation between urinary kinin

excretion and the time interval between micturitions supports the concept that degradation of kinins in the lower urinary tract is of minor importance and that urinary kinin excretion is a good indicator of intrarenal kinin formation. Furthermore, kinins are stable at room temperature for 24 h in urine acidified with glacial acetic acid and the generation of methionylsystylbradykinin by uropepsin (Hual, Kesser & Pisano 1976) appears to be minimal under these conditions. The level of kinin excretion in 4 hour urine found in this study is very similar to that reported by other investigators using radioimmunoassay (Mashford & Roberts 1971; Shimamoto et al. 1978) or bioassay (Jensen, Vennørd & Ruvik 1965; Mashford & Roberts 1971). Lower values have, however, also been reported (Abe et al. 1965; Hual, Keiser & Pisano 1976; Vinci et al. 1978). The reason for these differences is not clear.

The finding of positive correlations between urinary kinin excretion and natriuresis, kaluresis and diuresis is compatible with the concept that kinins play a physiological role in renal handling of electrolytes and water. This hypothesis was presented by Adetuyibi & Mills (1972) who found a positive correlation between urinary excretion of kallikrein and sodium in normal subjects on a free salt intake and an increase in urinary kallikrein excretion after a water load. Direct relations between urinary kallikrein excretion and natriuresis and diuresis have been found in normal subjects by Abe et al. (1978) and Hultén et al. (1979) but not by Margolius et al. (1974). Abe et al. (1978) also found that urinary kinin excretion was positively correlated to natriuresis and diuresis.

It has, however, been reported that urinary kallikrein excretion increased during sodium depletion in normal man (Margolius et al. 1974; Vinci et al. 1979). This increase was inhibited by spironolactone and is thus most likely due to enhanced aldosterone secretion (Margolius et al. 1974). Increased urinary kallikrein excretion has also been found in primary aldosteronism and in Bartter's syndrome (Margolius et al. 1971; Vinci et al. 1978). In these conditions urinary kinin excretion was, however, low-normal (Abe 1965; Vinci et al. 1978; Hultén et al. 1977) suggesting that the increase in urinary kallikrein excretion induced by aldosterone does not reflect enhanced intrarenal kinin generation. This is compatible with the report of Vinci et al. (1979) who found a decrease in urinary aldo-

sterone and kallikrein excretion in normal subjects changing from very low to high sodium intake which was not accompanied by any alteration in urinary kinin excretion.

Vinci et al (1979) observed no differences with regard to urinary excretion of kallikrein and kinins in normal subjects equilibrated on normal as compared to high sodium intake. All individuals were however on a fixed fluid intake of 2.5 l/day. Experimental data indicate that augmented sodium intake stimulates the renal kallikrein-kinin system only when fluid intake could increase to allow the expansion of some critical fluid volume, possibly the extracellular fluid volume (Marin-Grez & Carretero 1973; Mills & Ward 1975). The fixed fluid regime may thus be the reason for the lack of differences in urinary excretion of kallikrein and kinins on normal as compared to high sodium intake in this study.

The present finding of positive correlations between urinary kinins and urinary potassium and creatinine seems consistent with data in the epidemiological study by Zinner et al (1976). On the other hand Vinci et al (1979) found that a change from high to low potassium intake in normal subjects was associated with a decrease in urinary kallikrein excretion but no change in urinary kinin excretion. As the decrease in urinary kallikrein excretion was closely related to a reduction in aldosterone excretion, it probably did not reflect diminished intrarenal kinin generation.

The lack of a direct correlation between urinary aldosterone and urinary kinins in the present study supports the concept that aldosterone does not increase intrarenal kinin formation. The lack of significant correlations between urinary aldosterone and natriuresis and kaliuresis is in accordance with the findings of Tolhagen & Karlberg (1978) in 170 ambulatory normal individuals. The positive correlation observed between urinary noradrenaline and aldosterone suggests that aldosterone secretion is to some extent under the influence of the sympathetic nervous system, probably mediated by an increase in renin release (Gordon et al 1967).

Whereas urinary noradrenaline and adrenaline mainly reflect the general sympathetic activity, urinary dopamine is chiefly of renal origin (v. Euler, Fl  ding & Lishajko 1959; Alexander et al 1974). When given i.v. dopamine increases urinary sodium excretion (McDonald et al 1964) and previous studies have shown a direct relationship be-

tween urinary dopamine and natriuresis (Cuche et al 1972; Ball & Lee 1977). This was confirmed in the present study. Furthermore, there was a positive correlation between urinary kinin and dopamine. A jointly vasodilatory and natriuretic action of dopamine and kinins in the kidney has been suggested (Kuchel, Bau & Unger 1970). When infused into the renal artery of dogs, dopamine increased urinary kallikrein excretion in association with a natriuresis (Mills & Olicka 1976). The correlations between urinary dopamine and urinary noradrenaline and adrenaline previously found indicate that renal dopamine release is positively influenced by the sympathetic activity in normal or ambulatory individuals on a free salt and water intake.

A positive correlation between urinary noradrenaline and sodium may seem contradictory to other reports of lower urinary noradrenaline on high as compared to very low sodium intake (Gordon et al 1967; Alexander et al 1974). On the other hand Cuche et al (1972) did not find a decrease in urinary noradrenaline or adrenaline on very low as compared to normal sodium intake. Mills & Olicka (1977) reported that infusion of noradrenaline into the renal artery of the dog in a non-vasoconstrictor dose induced a small increase in urinary excretion of sodium, potassium and water. It is possible that a direct relationship between urinary noradrenaline and natriuresis is lost in sodium depletion due to an increase in sympathetic activity by a decline in plasma volume (Kelsch et al 1971).

The inverse relationship observed between urinary kinin excretion and age is consistent with the early finding of Elliot & Nuzum (1934) of decreasing urinary kallikrein excretion with age in 50 subjects with normal blood pressure.

The technical assistance of Mrs Gun or Johannev  r, Miss Barbro S  berg, Mrs Britt Louve S  nberg and Mr Thor Borge and the assistance with the preparation of the manuscript by Mrs Ann-Britt Nordvi is greatly acknowledged. This work was supported by grants from the Swedish National Association against Heart and Circulatory Diseases and from Squibb AB.

REFERENCES

- ABE, K. 1965 Urinary excretion of kinin in man with special reference to its origin. *Tohoku J. Exp. Med.* **87**, 175-184.
- ABE, K., IROKAWA, N., YASUJIMA, M., SEINO, M., CHIBA, S., SAKURAI, Y., YOSHINAGA, K. & SAITO, T. 1978 The kallikrein-kinin system and prostaglandin in the kidney in relation to furosemide.

- Reduced diuresis and to the renin-angiotensin-aldosterone system in man. *Circulat Res* 43: 234-260
- ETUYUBA, A. & MILLS, I. H. 1972. Relation between urinary kallikrein and renal function, hypertension, and excretion of sodium and water in man. *Lancet* 353: 357
- EXANDER, R. W. GILL, J. R., JR. & YAMABE, H. LOVENBERG, W. & KEISER, H. R. 1974. Effects of dietary sodium and of acute saline infusion on the interrelationship between dopamine excretion and adrenergic activity in man. *J Clin Invest* 54: 194-200
- ITON, A. H. & SAYRE, D. F. 1964. The distribution of dopamine and dopa in various tissues and a method for their determination in diverse biological material. *J Pharmacol Exp Ther* 145: 326-336
- LL, S. G. & LEE, M. R. 1977. The effect of carbidopa administration on urinary sodium excretion in man. I. Dopamine in intracerebral norepinephrine. *Brit J Clin Pharmacol* 4: 115-119
- RONE, F. A., PULLMAN, T. N., OPARIL, S. & YAKAMURA, S. 1976. Microspectroscopic evidence of rapid hydrolysis of bradykinin by rat proximal tubule. *Amer J Physiol* 230: 1430-1434
- RETERO, O. A. & SCICLI, A. G. 1976. Renal kallikrein: its localization and possible role in renal function. *Fed Proc* 35: 194-196
- RETERO, O. A., OZA, N. B., PIWONSKA, A., OCHOLIK, T. & SCICLI, A. G. 1976. Measurement of urinary kallikrein activity by kinetic radioimmunoassay. *Biochem Pharmacol* 25: 2265-2270
- KHE, J. L., KUCHEL, O., BARBEAU, A., BOUCHER, R. & GENEST, J. 1972. Relationship between the adrenergic nervous system and renal drug adaptation to upright posture: possible role for 3,4-dihydroxyphenethylamine (dopamine). *Can Sci* 47: 471-481
- BONO, E. & MILLS, I. H. 1974. Simultaneous increases in kallikrein in renal lymph and urine during saline infusion. *J Physiol (Lond.)* 41: 127P-128P
- ME, C. R. & CARVALHO, I. F. 1963. A micromethod for determination of bradykininase under several conditions. *Ann N Y Acad Sci* 104: 77-89
- SEN, V. 1964. Effect of hexamethylen bromide on plasma kallikrein formation, hydrolysis of p-tyrosyl-L-aspartyl-methyl ester and fluorimetry. *Brit J Pharmacol* 22: 87-103
- ITON, A. H. & NUZUM, F. R. 1934. The urinary excretion of a depressor substance (kallikrein of Frey and Kravt) in arterial hypertension. *Endocrinology* 18: 463-474
- ON, EULER, U. S. & FLODING, I. 1955. Fluorimetric estimation of noradrenaline and adrenaline in urine. *Acta Physiol Scand* 33 (Suppl. 118): 57-62
- ON, EULER, U. S. & FLODING, I. & LISHAJKO, F. 1979. The presence of free and conjugated 3,4-dihydroxyphenylacetic acid (dopa) in urine and blood plasma. *Acta Societatis Medicorum Upsalienis* 64: 217-223
- OL, R. H., GOLDSMITH, R., KIDD, B. J. & LEWIS, C. P. 1961. Bradykinin as a vasodilator in man. *J Physiol (Lond.)* 157: 549-562
- REKER, R., MANN, K. & BETTELS, T. 1977. Isolation of human urinary kallikrein by affinity chromatography. *J China Clin Biochem* 15: 479-483
- GORDON, R. D., KUCHEL, O., LIDDLE, G. W. & ISLAND, D. B. 1967. Role of the sympathetic nervous system in regulating renal and aldosterone production in man. *J Clin Invest* 46: 599-605
- HALUSHKA, P. V., MARGOLIS, H. S., ALLEN, H. & CONRAD, E. C. 1979. Urinary excretion of prostaglandin E like material and kallikrein: effects of flurosteroid. *Prostaglandins* 18: 359-368
- HAMBERG, U. 1965. Isolation of bradykinin from human plasma. *Ann Academiæ Scientiarum Fennicæ. Ser. A II. Chemica* 113
- HIAL, V., KEISER, H. R. & PISANO, J. J. 1976. Origin and content of methionyl-histyl-bradykinin, lysyl-bradykinin and bradykinin in human urine. *Biochem Pharmacol* 25: 299-303
- HULTHÉN, U. L. & BORGE, T. 1976. Determination of bradykinin in blood by sensitive radioimmunoassay. *Scand J Clin Lab Invest* 36: 873-879
- HULTHÉN, U. L., DYMLING, J. F., HANSSON, B. G. & HÖKFELT, B. 1977. Effects of indomethacin (Coford®) on plasma renin, angiotensin II, aldosterone, catecholamines and electrolytes in Bartter's syndrome. *Acta Endocr (Kbh)* 87 (Suppl. 212): 93
- ITO, T., WOO, J., HANING, R. & HORTON, R. 1972. A radioimmunoassay for aldosterone in human peripheral plasma including comparison of alternate techniques. *J Clin Endocr Metab* 34: 106-111
- JENSEN, K., BRUSEID, VENNERÖD, A. M. & RYVITK, S. F. 1965. Excretion of human urinary kinins. *Acta Pharmacol Toxicol* 22: 177-186
- KELSCH, R. C., LIGHT, G. S., LUCIANO, J. R., OLIVER, W. J. & VADNAY, L. 1971. The effect of prednisone on plasma norepinephrine concentration and renal activity in salt-depleted man. *J Lab Clin Med* 77: 267-277
- KRAMER, H. J., MOCH, T. & VON SICKERER, L. 1978. Effects of inhibition of the kallikrein system on renal hemodynamics and salt and water excretion in actively saline-loaded rats. *Acta Endocr (Kbh)* 87 (Suppl. 133): 134
- KUCHEL, O., BUU, N. T. & UNGER, T. 1978. Dopamine-sodium relationship: I. Dopamine: part of the endogenous natriuretic system. *Contr Nephrol* 13: 27-36
- LEVINSKY, N. G., OLÉ-MOYOL, O., AUSTEN, K. F. & SPRAGG, J. 1979. Measurement of human urinary kallikrein and evidence for non-kallikrein urinary TAM esterase by direct immunoassay and by affinity chromatography. *Biochem Pharmacol* 28: 491-495
- LOUHIJA, A. & SIMILÄ, R. 1971. Plasma kininogen determination by use of crude and purified trypsin hydrolyzates. *Acta Pharmacol Toxicol* 30: 122-128
- MARGOLIS, H. S., GELLER, R., PISANO, J. J. & SJÖERDSEMA, A. 1971. Altered urinary kallikrein excretion in human hypertension. *Lancet* 2: 1063-1065
- MARGOLIS, H. S., HORWITZ, D., GELLER, R. G., ALEXANDER, R. W., GILL, J. R. & PISANO, J. J. & KEISER, H. R. 1974. Urinary kallikrein excretion

sterone and kallikrein excretion in normal subjects changing from very low to high sodium intake which was not accompanied by any alteration in urinary kinin excretion.

Vinci et al (1979) observed no differences with regard to urinary excretion of kallikrein and kinins in normal subjects equilibrated on normal as compared to high sodium intake. All individuals were however on a fixed fluid intake of 2.5 l/day. Experimental data indicate that augmented sodium intake stimulates the renal kallikrein-kinin system only when fluid intake could increase to allow the expansion of some critical fluid volume, possibly the extracellular fluid volume (Marin-Grez & Carretero 1973; Mills & Ward 1975). The fixed fluid regime may thus be the reason for the lack of differences in urinary excretion of kallikrein and kinins on normal as compared to high sodium intake in this study.

The present finding of positive correlations between urinary kinins and urinary potassium and creatinine seems consistent with data in the epidemiological study by Zinner et al (1976). On the other hand Vinci et al (1979) found that a change from high to low potassium intake in normal subjects was associated with a decrease in urinary kallikrein excretion but no change in urinary kinin excretion. As the decrease in urinary kallikrein excretion was closely related to a reduction in aldosterone excretion, it probably did not reflect diminished intrarenal kinin generation.

The lack of a direct correlation between urinary aldosterone and urinary kinins in the present study supports the concept that aldosterone does not increase intrarenal kinin formation. The lack of significant correlations between urinary aldosterone and natriuresis and kaliuresis is in accordance with the findings of Tohagen & Karlberg (1978) in 170 ambulatory normal individuals. The positive correlation observed between urinary noradrenaline and aldosterone suggests that aldosterone secretion is to some extent under the influence of the sympathetic nervous system, probably mediated by an increase in renin release (Gordon et al 1967).

Whereas urinary noradrenaline and adrenaline mainly reflect the general sympathetic activity, urinary dopamine is chiefly of renal origin (v. Euler, Floding & Lshajko 1959; Alexander et al 1974). When given i.v. dopamine increases urinary sodium excretion (McDonald et al 1964) and previous studies have shown a direct relationship be-

tween urinary dopamine and natriuresis (Cuche et al 1977; Ball & Lee 1977). This was confirmed in the present study. Furthermore, there was a positive correlation between urinary kinins and dopamine. A jointly vasodilatory and natriuretic action of dopamine and kinins in the kidney has been suggested (Kuchel, Bar & Unger 1978). When infused into the renal artery of dogs, dopamine increased urinary kallikrein excretion in association with a natriuresis (Mills & Obika 1976). The correlations between urinary dopamine and urinary noradrenaline and adrenaline presently found indicate that renal dopamine release is positively influenced by the sympathetic activity in normal or bulimic individuals on a free salt and water intake.

A positive correlation between urinary noradrenaline and sodium may seem contradictory to earlier reports of lower urinary noradrenaline on high as compared to very low sodium intake (Gordon et al 1967; Alexander et al 1974). On the other hand, Cuche et al (1977) did not find a decrease in urinary noradrenaline or adrenaline on very low as compared to normal sodium intake. Mills & Obika (1977) reported that infusion of noradrenaline into the renal artery of the dog in a non-vasoconstrictor dose induced a small increase in urinary excretion of sodium, potassium and water. It is possible that a direct relationship between urinary noradrenaline and natriuresis is lost in sodium depletion due to an increase in sympathetic activity by a decrease in plasma volume (Kelsch et al 1971).

The inverse relationship observed between urinary kinin excretion and age is consistent with the early finding of Elliot & Nuzum (1974) of decreased urinary kallikrein excretion with age in 50 subjects with normal blood pressure.

The technical assistance of Mrs Gun or Johansson, Miss Barbro Sjöberg, Mrs Britt Loubé, B. Enberg and M. Thor Borge and the assistance with the preparation of the manuscript by Mrs Ann-Britt Nordvi is greatly acknowledged. This work was supported by grants from the Swedish National Association against Heart and Circulatory Diseases and from Squibb AB.

REFERENCES

- ABE K. 1963 Urinary excretion of kinin in man: a special reference to its origin. *Tohoku J. Exp. Med.* 87: 175-184.
- ABE K., IROKAWA M., YASUJIMA M., SEINO M., CHIBA M., SAKURAI Y., YOSHINAGA K. & SAITO T. 1978 The kallikrein-kinin system and prostaglandin in the kidney: Their relation to furosemide.

- selected desensitization and to the renin-angiotensin-aldosterone system in man. *Circulat Res* 43: 254-260.
- TUOHIM A. & MILLS I. H. 1972 Relation between urinary kallikrein and renal function, hypertension and excretion of sodium and water in man. *Lancet* 203: 207.
- XANDER, R. W. GILL, J. R. J. YAMABE, H. LOVENBERG W. & KEISER, H. R. 1974 Effect of acute sodium load and of acute sodium inhibition on the relationship between dopamine excretion and aldosterone activity in man. *J Clin Invest* 54: 194-200.
- TOY A. H. & SAYRE, D. F. 1964 The distribution of dopamine and dopamine in various animals and method of their determination in diverse biological material. *J Pharmacol Exp Ther* 145: 326-334.
- J. S. G. & LEE, M. R. 1977 The effect of carbidopa administration on urinary sodium excretion in man. *J Japanese of extrarenal antidiuretic hormones*. *Brit J Clin Pharmacol* 4: 115-119.
- ROY, F. A., PULLMAN T. N., OPARIL, S. & YAKAMURA S. 1976 Microperfusion evidence of rapid hydrolysis of bradykinin by rat peritoneal capillary. *Am J Physiol* 230: 1403-1404.
- KRETERO, O. A. & SCICLI, A. G. 1976 Renal kallikrein as an ionization and possible role in renal function. *Fed Proc* 35: 194-196.
- KRETERO, O. A., OZA, N. B., PIWONSKA A., DOHOLIK, T. & SCICLI, A. G. 1976 Measurement of urinary kallikrein activity by laser radioimmunoassay. *Rechen Pharmacol* 25: 2265-2270.
- CHE, J. L., KUCHEL, O., BARBEAU A., BOUCHER, R. & GENEST J. 1972 Relationship between the adrenergic nervous system and renal during adaptation to upright posture: possible role for 3,4-dihydroxyphenylethylamine (dopamine). *Clin Sci* 43: 411-419.
- BOYD E. & MILLS, I. H. 1974 Somatomedin secreted in kallikrein in renal lymph and urine during saline infusion. *J Physiol (Lond)* 241: 127P-128P.
- NIZ, C. R. & CARVALHO J. F. 1963 A micro-method for determination of bradykininogen under several conditions. *Ann N Y Acad Sci* 104: 77-89.
- SEN, V. 1964 Effect of benzodrine hydrochloride on plasma kallikrein formation, hydrolysis of p-tosyl-L-arginine methyl ester and fibrinolytic. *Brit J Pharmacol* 22: 87-103.
- JUST A. H. & MUZYM F. R. 1934 The primary excretion of depressor substance (kallikrein of Frey and Kraus) in arterial hypertension. *Endocrinology* 18: 442-474.
- JOHNSON, U. S. & FLOODING I. 1955 Fluorimetric estimation of noradrenaline and adrenaline in urine. *Acta Physiol Scand* 33: Suppl. 118: 37-42.
- JOHNSON, U. S., FLOODING I. & LISHAJKO F. 1959 The presence of free and conjugated 3,4-dihydroxyphenylacetic acid (dopac) in urine and blood plasma. *Acta Societatis Medicorum Upsalensis* 64: 217-225.
- OL, R. H., GOLDSWITH R., KIDD D. J. & LEWIS, G. P. 1961 Bradykinin as a vasodilator in man. *J Physiol (Lond)* 117: 599-602.
- SEGER, R., MANN K. & BETTELS, T. 1977 Isolation of human urinary kallikrein by affinity chromatography. *J Chem Clin Biochem* 15: 479-483.
- GORDON R. D., KUCHEL, O., LIDDLE, G. W. & ISLAND D. P. 1967 Role of the sympathetic nervous system in regulating renal and aldosterone production in man. *J Clin Invest* 46: 599-605.
- HALUSHKA, P. V., MARGOLIUS, H. S., ALLEN H. & CONRADI E. C. 1979 Urinary excretion of prostaglandin E like material and kallikrein effects of furosemide. *Prostaglandins* 18: 359-366.
- HAMBERG, U. 1962 Isolation of bradykinin from human plasma. *Ann Academiæ Scientiarum Fennicæ. Ser. A. II. Chemica* 113.
- HIAL, V., KEISER, H. R. & PISANO J. J. 1976 Origin and content of acetyl-L-histyl-bradykinin, lysyl-bradykinin and bradykinin in human urine. *Biochem Pharmacol* 31: 2499-2503.
- HULTHÉN U. L. & BORGE, T. 1976 Determination of bradykinin in blood by sensitive radioimmunoassay. *Scand J Clin Lab Invest* 36: 833-839.
- HULTHÉN U. L., DYLLING, J. F., HANSSON B. G. & HÖKFELT B. 1977 Effects of isometheptate (Cofort®) on ketans, PRA, angiotensin II, aldosterone, catecholamines and electrolytes in Bartter's syndrome. *Acta Endocr (Kbh)* 85: Suppl. 212-97.
- ITO T., WOO J., HANING R. & HORTON R. 1977 A radioimmunoassay for aldosterone in human peripheral plasma including comparison of alternate techniques. *J Clin Endocr Metab* 34: 106-112.
- JENSEN K., BRISID, VENNED A. H. & RIVNIK S. F. 1965 Excretion of human urinary ketans. *Acta Pharmacol Toxicol* 22: 177-186.
- KELCH R. C., LIGHT G. S., LUCIANO J. R., OLIVER, W. J. & VADNAY L. 1971 The effect of prednisone on plasma renin-angiotensin concentration and renal activity in salt-depleted man. *J Lab Clin Med* 77: 267-277.
- KRAMER, H. J., MOCH T. & VON SICHERER, L. 1978 Effect of inhibition of the lutein system on renal hemodynamics and salt and water excretion in acutely saline-loaded rats. *Acta Endocr (Kbh)* 87: Suppl. 215: 133-134.
- KUCHEL, O., RUU M. T. & UNOER, T. 1978 Dopamine-aldosterone relationship. I. dopamine part of the endogenous antidiuretic system. *Cover Nephrol* 13: 27-36.
- LEVINSKY M. G., OLE-MOYOH O., AUSTEN K. F. & SPRAOG J. 1979 Measurement of human urinary kallikrein and evidence for non-kallikrein urinary TAME esterase by direct immunoassay and by affinity chromatography. *Biochem Pharmacol* 28: 2491-2495.
- LOUHIA A. & SIPIA, R. 1971 Plasma kallikrein determination by use of crude and purified trypan hydrolytic. *Acta Pharmacol Toxicol* 30: 122-128.
- MARGOLIUS, H. S., GELLER R., PISANO J. J. & SJÖERDSMA, A. 1971 Altered urinary kallikrein excretion in human hypertension. *Lancet* 2: 1063-1065.
- MARGOLIUS H. S., HORWITZ, D., GELLER, R. O., ALEXANDER, R. W. GILL, J. R. J., PISANO J. J. & KEISER, H. R. 1974 Urinary kallikrein excretion

- in normal man. Relationships to sodium intake and sodium-retaining steroids. *Circulat Res* 35: 812-819.
- MARIN-GREZ M 1974 The influence of antibodies against bradykinin on isotonic saline diuretics in the rat. *Pflügers Arch* 330: 231-239.
- MARIN-GREZ M & CARRETERO O A 1972. A method for measurement of urinary kallikrein. *J Appl Physiol* 33: 428-431.
- MARIN-GREZ M & CARRETERO O A 1973 The relationship between urinary kallikrein and natriuresis. In: *Kininogenases, kallikrein. 1st Symposium on Physiological Properties and Pharmacological Rational* 1972 (ed G. L. Haberland & J. W. Rohen) pp 113-122. F. K. Schattauer Verlag, Stuttgart and New York.
- MASHFORD M L & ROBERTS M L 1971 Determination of human urinary kinin levels by radioimmunoassay using a tyrosine analogue of bradykinin. *Biochem Pharmacol* 20: 969-973.
- McCONNELL D J 1977 Inhibitors of kallikrein in human plasma. *J Clin Invest* 51: 1611-1613.
- McDONALD R H Jr, GOLDBERG L I, McNAY J L & TUTTLE E P Jr 1964 Effects of dopamine in man. Argumentation of sodium excretion, glomerular filtration rate, and renal plasma flow. *J Clin Invest* 43: 1116-1124.
- MILLS I H & OBIKA L F O 1976 The effect of adrenergic and dopamine receptor blockade on the kallikrein and renal response to intra-arterial infusion of dopamine in dogs. *J Physiol* 263: 150P-151P.
- MILLS I H & OBIKA L F O 1977 A novel effect of intrarenal infusion of a non-vasoconstrictor dose of noradrenaline on renal function: relationship to renal kallikrein and prostaglandin. *J Physiol* 267: 1P-2P.
- MILLS I H & WARD P E 1975 The relationship between kallikrein and water excretion and the conditional relationship between kallikrein and sodium excretion. *J Physiol (Lond)* 46: 695-707.
- MILLS I H, MACFARLANE N A A, WARD P E & OBIKA L F O 1976 The renal kallikrein-kinin system and the regulation of salt and water excretion. *Fed Proc* 35: 181-188.
- MUSTAD A, VAAJE A & PIERCE J V 1975 Synthesis of kallikreins by rat kidney slices. *Brit J Pharmacol* 53: 229-234.
- PISANO J J, YATES A & PIERCE, J V 1978 Kininogen in human urine. *Agents and Actions* 8: 153.
- ROBLERO J, CROYATTO H, GARCIA R, CORTHORN J & DE VITO E 1976. Kallikrein-like activity in perfusates and urine of isolated rat kidneys. *Amer J Physiol* 231: 1383-1389.
- ROUTH J I, BANNOW III E, FINCHAM R. W & STOLL, J. L. 1971 Excretion of L-dopa and its metabolites in urine of Parkinson's disease patients receiving L-dopa therapy. *Clin Chem* 17: 867-871.
- SCICLI A G, GANDOLFI R. & CARRETERO O A 1978 Site of formation of kinins in the dog. *Amer J Physiol* 234: F36-F40.
- SCICLI A G, CARRETERO O A, HAMPTON A, CORTES P & OZA N B 1976 Site of kininogen secretion in the dog nephron. *Amer J Physiol* 230: 533-536.
- SEALY J E, BUEHLER F R, LARAGH J H, MANNING E L & BRUNNER, H R 1977 Steroid excretion. Physiological variations in man measured by radioimmunoassay or double-isotope dilution. *Circulat Res* 31: 367-378.
- SHIMAMOTO K, ANDO T, NAKAO T, TANAKA S, SAKUMA M & MIYAHARA M 1978 A sensitive radioimmunoassay method for urinary kinins. *J Lab Clin Med* 91: 721-728.
- STÜRMER E & HERDE, B. 1966 Kallikrein-Bradykinin: vergleichende pharmakologische Untersuchungen. Naunyn-Schmiedeberg's Arch Exp Pathol Pharmacol 43: 355-366.
- TOLHAGEN K & KARLBERG B E 1978 Renin and urinary aldosterone and their interrelations to blood pressure, plasma renin activity and urinary electrolytes in normotensive subjects. *Scand J Clin Invest* 38: 741-747.
- VINCI J M, ZUSMAN R, MIZZO J L, & BOWDEN R E, HORWITZ, D, PISANO J J & MIZZO J L 1979 Human urinary and plasma kinin. Relationship to sodium-retaining steroids and plasma renin activity. *Circulat Res* 44: 28-37.
- VINCI J M, GILL, J R, Jr, BOWDEN R E, PISANO J J, MIZZO J L, Jr, RADFALL, V, TAYLOR A A, ZUSMAN R, MIZZO J L, C & KEISER H R 1978 The kallikrein-kinin system in Bartter's syndrome and its response to prostaglandin synthetase inhibition. *J Clin Invest* 61: 167-168.
- WEBSTER M E & PIERCE J V 1963 The nature of the kallikrein released from human plasma by kallikrein and other enzymes. *Ann N Y Acad Sci* 104: 91-107.
- WEBSTER M E & GILMORE J P 1964 Influence of kallikrein-10 on renal function. *Amer J Physiol* 206: 714-718.
- WERLE E & MAIER L 1952 Über die chemische und pharmakologische Unterscheidung von Kallikreinen verschiedener Herkunft. *Biochem Z* 233: 279-283.
- WERLE P & ZACH P 1970 Verteilung von Kallikrein in Serum und Geweben bei Ratten und anderen Säugetieren. *Z Klin Chem Klin Biochem* 8: 186-189.
- ZINNER S H, MARGOLIS H S, ROSEN, KEISER H R & KASS E H 1976 Familial hyperkalemia. Relation to blood pressure, race and electrolytes. *Amer J Epidemiol* 104: 1-132.

Central interaction between reflex responses to activity in aortic nerve A- and C-fibres

RALD AARS

Department of Physiology and Biochemistry, Dental Faculty,
University of Oslo, Box 1052, Oslo, Norway

AARS, H.: Central interaction between reflex responses to activity in aortic nerve A- and C-fibres. *Acta Physiol Scand* 1980, 110: 315-320. Received 10 March 1980. (ISSN 0001-6772). Department of Physiology and Biochemistry, Dental Faculty, University of Oslo, Norway.

The question of whether or not activity in myelinated (A) and non-myelinated (C) afferents in baroreceptor nerve show interaction of their reflex effects, was investigated in 10 anesthetized rabbits. The central end of the cut left aortic nerve was stimulated by two sets of electrodes, one for selective high-frequency excitation of A-fibres (A-stimulation) and one for low-frequency activation of C- (and A-) fibres by another pulse generator (C-stimulation). The pulse rates in C-stimulations were too low to evoke reflex effects via A-fibres. The hypotensive response to combined stimulations of A- and C-fibres (AC-stimulation) was found to exceed the sum of responses to separate A- and C-stimulations in 21 of 22 stimulation series. For sympathetic activity to the kidney, greater than additive effect as observed in 16 of 24 series ($P=0.06$). While in 6 of the series, the responses were equal. Median values of the ratio AC/(A+C) were 1.28 for the reflex changes in pressure and 1.11 for the effects on renal nerve activity. Similarly, C-stimulation which in comparison to A-stimulation affected sympathetic activity relatively more than blood pressure, in 13 of 16 series ($P=0.01$) produced a greater sympathetic inhibition when added to background of A-fibre activity than when alone. These results suggest that synergistic interaction exists between central effects of afferent discharge in aortic nerve A- and C-fibres.

Key words: Aortic nerve electrical stimulation, myelinated and non-myelinated afferents, baroreceptor reflex, arterial pressure, sympathetic activity.

Recent work on the response characteristics of baroreceptor C-fibres in rabbits and rats has shown that the C-fibre receptors have a higher pressure threshold than the A-fibre receptors and a lower discharge frequency (Jones & Thorén 1977, Thorén & Jones 1977, Thorén, Saum & Brown 1977). In the rabbit, they contribute to the baroreflex response to abrupt rise in pressure only when mean pressure increased by more than 20 mmHg from normal resting pressures, to levels above 110 mmHg (Aars, Sylter & Haswell 1978).

The present study is an attempt to examine another aspect of A- and C-fibre participation in the baroreflex: the question of whether reflex effects of activity in the two types of fibres are subject to simple addition, or whether a synergistic or inhibitory central interaction exists between the two. To

this end, I compared the reflex responses to electrical stimulation of aortic nerve A- or C-fibres to that of A- and C-fibres combined, in anesthetized rabbits. As parameters of the reflex I used changes in arterial blood pressure and sympathetic activity to the kidney.

METHODS

The experiments were performed in 10 rabbits, of body weights 3.0-3.9 kg. For anesthesia, they received an injection of Hypnorm (AB Miskos, 0.10-0.15 ml/kg), followed by mixture of chloralose (0.01 g/kg) and urethane (0.75 g/kg) half and half *p*. Urethane was supplemented when necessary. The animals were tracheotomized and respired air. Blood pressure was measured with transducer (Statham P23Dc) connected to catheter in the left femoral artery. Efferent sympathetic activity was recorded in the left renal nerve by means

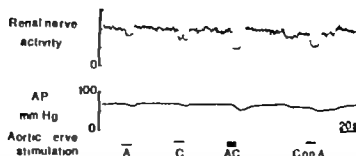


Fig. 1 Reflex changes in average renal nerve activity and mean blood pressure (AP) in response to separate and combined stimulation of A and C fibres in the left aortic nerve. A: A fibre stimulation with pulses of 0.05 ms, 6 V, 60 Hz in heart synchronous trains of 80 ms duration. C: Effects via C fibres during continuous stimulation with pulses of 1 ms, 6 V, 4 Hz. AC: The same stimulation characteristics as in A and C but applied simultaneously. Con A: C stimulation started 30 s after onset of A stimulation. For both reflex parameters, $AC > (A+C)$ responses and $Con A > C$.

of silver electrodes and a differential amplifier (Grass P15). The nerve was cut distally. The activity was averaged (Coupler 985-A) and recorded on a Beckman R 411 Dynograph together with pulse width and mean arterial pressure and heart rate. Output from the averager was linearly related to frequency of input signal within the range of activity observed in the renal nerve. The heart rate was obtained from a tachometer (Coupler 9857B) which was triggered through a special circuit by the rising phase of the arterial pressure. The same circuit could also be used to trigger the pulse generators (Grass SD9 and S48) used for stimulation of the left aortic nerve. This nerve was cut low in the neck and placed on two pairs of silver electrodes, one for A fibre stimulation (pulses of 6 V, 0.05 ms duration and 60–100 Hz in trains of 80–100 ms duration), the other for stimulation of C-fibres (6 V, 1 ms, 4 Hz continuous stimulation or in two animals, one pulse every second or fifth heart beat triggered with a fixed 5 ms delay).

Stimulation periods were 10 or 30 s with intervals of about 1 min. In each period one of the following types of stimulation was applied: (1) A-fibre stimulation, high frequency (marked A); (2) C-fibre stimulation, low frequency (marked C); (3) A and C fibre stimulation combined (marked AC); (4) C-fibre stimulation added 10–45 s after start of A fibre stimulation (marked C on A). The stimulations were usually repeated once or twice in variable sequence in order to obtain average responses. Complete series consisting of these 4 types of stimulation were run for several different stimulation characteristics in each animal.

The pulse duration used for A stimulation was below threshold for excitation of C-fibres but during C stimulation A-fibres were also activated. Classification of reflex effects as due to separate activation of A and C-fibres in A and C stimulation respectively is based on the fact that at the low frequency used in C stimulation A-fibre discharge is devoid of reflex actions (Dougherty, Ritchie & Schaumann 1956; Kardon, Peterson & Bishop 1973; Akre & Aars 1977; Aars, Myhre & Haswell 1978).

The results were read as maximum reductions in blood pressure and sympathetic activity (mm on recording

paper) and mean value were usually compared directly or as the ratio $AC/(A+C)$. For comparison of reflex changes evoked by A and C-fibre activation, the relations in blood pressure and sympathetic discharge were expressed in per cent of the immediately preceding activity. Zero renal nerve activity was determined as level of the electrical noise recorded at the end of experiment after the nerve had been cut cranially but remained in contact with the tissues.

Experiment included in this presentation were limited to those in which (a) control blood pressure were above 90 mmHg mean, 60 mmHg diastolic; (b) the mean of 4 responses in each series was less than 25 mmHg and reduction in renal nerve activity and the control pressure responses did not reach mean pressure level below 90 mmHg; (c) responses remained unaltered throughout the experiment.

RESULTS

Reflex responses to combined stimulation of A and C fibre (AC) usually exceeded the summed responses to separate A and C fibre stimulation. This is shown for a typical experiment in Fig. 1 and for the whole material in Figs. 2 and 3. For the blood pressures, $AC > (A+C)$ was found in 1 of 2 stimulation series ($P < 0.01$). Equivalent figures for the reduction in renal nerve activity were 16 of 17 ($P = 0.08$). Average ratios of $AC/(A+C)$ (median, 5–75 percentiles) were 1.78 (1.08–1.57) and 1.10 (1.00–1.70) respectively. Intra-individual variations in reflex responses were small as shown by observations in three animals, in which mean responses were based on 3–4 consecutive A and AC stimulations. In these series individual readings differed from the calculated mean values by less than 2 mm for sympathetic and 3 mmHg for pressure responses.

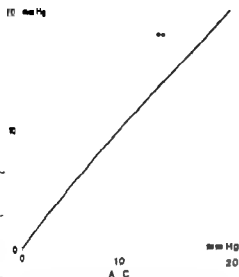


Fig. 2. Reflex reductions in blood pressure. A comparison between the effects of combined stimulation (abscissa) and the sum of responses to separate stimulation (ordinate) of aortic nerve A- and C-fibres. Various stimulation characteristics. 22 stimulations in five rabbits. Cf. Methods for further details.

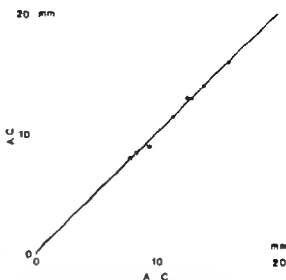


Fig. 3. Reflex inhibition of renal nerve activity (read as millimeter on recording paper). A comparison between effects of combined and separate stimulations of aortic nerve A- and C-fibres, plotted as in Fig. 2. 4 observations in 6 rabbits.

Reflex effects of "C" on A-stimulations were compared to the effects of C and AC-stimulations. 16 series in 9 rabbits. As shown by the series in Fig. 1 the sympathetic inhibitory effect of A-stimulation tended to decrease with time whereas the reflex response usually increased before the start of C-stimulation. This difference was related to the comparison of effects of "C" on A and AC-stimulations: The sums of the A-effects measured at the start of C-stimulation and the total C-effects were invariably smaller than the C-responses when sympathetic activity was excluded, and larger when calculated for the hypotensive responses. However C-stimulations were usually more effective when added to A-stimulations than when alone. Thus, in 16 series, the reduction in renal nerve activity was increased in 13 ($P < 0.01$) unaltered in two and reduced in one when C-stimulation was superimposed on A-fibre discharge. For reflex changes in blood pressure these figures were 7, 6 and 3 respectively. The reflex "C" on A+AC was 1.19 (median, 23-75 per centiles 1.10-1.26) for the sympathetic responses. It was not calculated for the effects on pressure since the responses of this parameter to C-stimulation were rather small (Fig. 5).

While excitation of A fibres by C-stimulation did not disturb the study of reflex effects of separate A and C-stimulations (cf. Methods) the "extra" A fibre activation during AC stimulation might coincide with the trains of A stimulation, thereby causing increased A-fibre discharge in a frequency range above threshold for reflex actions. Since the reflex effects of AC-stimulation surpassed the (A+C)-effects (Figs. 1-3) it was necessary to evaluate how much of the increase might have been due to the "extra" A-fibre activity. A typical example of this evaluation is shown in Fig. 4 in which the sympathetic reduction resulting from three series of A, C and AC stimulations have been plotted together with the calculated influence of the "extra" A fibre activity. A-stimulation was performed by heart synchronous trains of 80 Hz/80 ms duration, 80/100 or 100/100, equivalent to respectively 8 and 10 pulses per heart beat. With a heart rate of 320 beats/min, the constant 2 Hz C-stimulation will hit less than two A trains per second, equal to an average increase of about 0.3 A-impulses per heart beat. From the recorded responses to A-stimulation alone (Fig. 4) this addition can be expected to produce a further 1.2% reduction in sympathetic activity. As shown in this figure and supported

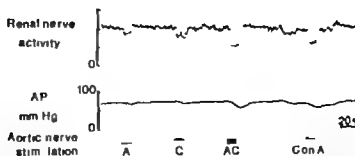


Fig. 1. Reflex changes in average renal nerve activity and mean blood pressure (AP) in response to separate and combined stimulation of A- and C-fibres in the left aortic nerve A: A-fibre stimulation with pulses of 0.0 ms, 6 V, 60 Hz in heart-synchronous trains of 80 ms duration. C: Effects via C-fibres, during continuous stimulation with pulses of 1 ms, 6 V, 4 Hz. AC: The same stimulation characteristics as in A and C but applied simultaneously. C on A: C stimulation started 30 s after onset of A stimulation. For both reflex parameters AC \sim (A + C) responses and C on A \sim C.

of silver electrodes and a differential amplifier (Grass P15). The nerve was cut distally. The activity was averaged (Coupler 945A) and recorded on a Beckman R 411 Dynograph together with pulsatile and mean arterial pressure and heart rate. Output from the agraph was linearly related to frequency of input signal within the range of activity observed in the renal nerve. The heart rate was obtained from a tachometer (Coupler 945TB) which was triggered through a special circuit, by the rising phase of the arterial pressure. The same circuit could also be used to trigger the pulse generators (Grass SD9 and S48) used for stimulation of the left aortic nerve. This nerve was cut low in the neck and placed on two pairs of silver electrodes, one for A-fibre stimulation (pulses of 6 V, 0.0 ms duration and 60–100 Hz in trains of 80–100 ms duration), the other for stimulation of C-fibres (6 V, 1 ms, 4 Hz continuous stimulation or in two animals, one pulse every second or fifth heart beat triggered with a fixed 5 ms delay).

Stimulation periods were 10 or 20 s with intervals of about 1 min. In each period one of the following types of stimulation was applied: (1) A-fibre stimulation, high frequency (marked A); (2) C-fibre stimulation, low frequency (marked C); (3) A- and C-fibre stimulation combined (marked AC); (4) C-fibre stimulation added 10–45 s after start of A-fibre stimulation (marked C on A). The stimulations were usually repeated once or twice in variable sequence in order to obtain average responses. Complete series consisting of these 4 types of stimulation were run for several different stimulation characteristics in each animal.

The pulse duration used for A stimulation was below threshold for excitation of C-fibres but during C stimulation A-fibres were also activated. Classification of reflex effects as due to separate activation of A- and C-fibres, A- and C stimulation respectively, is based on the fact that at the low frequency used in C stimulation A-fibre discharge is devoid of reflex actions (Douglas, Ritchie & Schaumann 1956; Kardon, Peterson & Bishop 1973; Aars & Aars 1977; Aars, Myhre & Haswell 1978).

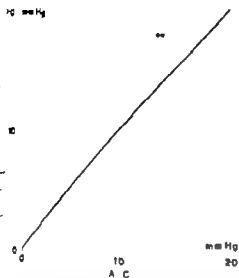
The results were read as maximum reduction in blood pressure and sympathetic activity (mm on recording

paper) and mean values were usually compared directly or as the ratio $AC/(A+C)$. For comparison of reflex changes evoked by A- and C-fibre activation, the reductions in blood pressure and sympathetic discharge were expressed in per cent of the immediately preceding control activity. Zero renal nerve activity was determined at the level of the electrical noise recorded at the end of experiments after the nerve had been cut cranially but remained in contact with the tissues.

Experiments included in this presentation were limited to those in which (a) control blood pressure was above 70 mmHg, mean 60 mmHg diastolic; (b) the sum of A- and C-fibre responses in each series was less than 25 mmHg and the reduction in renal nerve activity and the summed pressure responses did not reach mean pressure levels below 50 mmHg; (c) responses remained unaltered throughout the experiments.

RESULTS

Reflex responses to combined stimulation of A- and C-fibres (AC) usually exceeded the summed responses to separate A- and C-fibre stimulation. This is shown for a typical experiment in Fig. 1 and for the whole material in Figs. 2 and 3. For the blood pressures $AC > (A+C)$ was found in 71 of 22 stimulation series ($P < 0.01$), equivalent figures for the reduction in renal nerve activity were 16 of 16 ($P = 0.08$). Average ratios of $AC/(A+C)$ (median 1.25–75 percentiles) were 1.28 (1.08–1.55) and 1.10 (1.00–1.20) respectively. Intra-individual variations in reflex responses were small as shown by observations in three animals, in which mean responses were based on 3–4 consecutive A, C and AC stimulations. In these series individual responses differed from the calculated mean values less than 2 mm for sympathetic and 3 mmHg for pressure responses.



2. Reflex reductions in blood pressure. A comparison with line of identity between responses to combined stimulation (ordinates) and the sum of responses to separate stimulation (abscissa) of aortic nerve A- and C-fibres, various stimulation characteristics, 22 stimulations in 6 rabbits. Cf. Methods for further details.

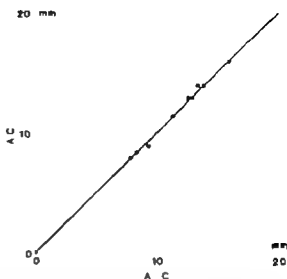


Fig. 3. Reflex inhibition of renal nerve activity (read in millimeter on recording paper). A comparison between effects of combined and separate stimulations of aortic nerve A- and C-fibres, plotted as in Fig. 2. 24 observations in 6 rabbits.

Reflex effects of "C" on A stimulations were compared to the effects of C and AC-stimulations in 16 series in 9 rabbits. As shown by the series in Fig. 1 the sympathetic inhibitory effect of A-stimulation tended to decrease with time whereas the reflexive response usually increased before the act of C-stimulation. This difference was reduced in the comparison of effects of "C" on A and AC-stimulations. The sums of the A-effects occurring at the start of C-stimulation and the added C-effects were invariably smaller than the C-responses when sympathetic activity was ignored, and larger when calculated for the hypotensive responses. However C-stimulations were really more effective when added to A-stimulations than when alone. Thus, in 16 series, the reduction in renal nerve activity was increased in 13 (P=0.01), unchanged in two and reduced in one. C-stimulation was superimposed on A-fibre discharge. For reflex changes in blood pressure the figures were 7, 6 and 3 respectively. The "C" on A/C was 1.19 (median 25-75 per centiles 1.0-1.26) for the sympathetic responses. It was not calculated for the effects on pressure since the responses of this parameter to C-stimulation are rather small (Fig. 5).

While excitation of A-fibres by C-stimulation did not disturb the study of reflex effects of separate A- and C-stimulations (cf. Methods) the extra A-fibre activation during AC stimulation might coincide with the trains of A stimulation thereby causing increased A-fibre discharge in a frequency range above threshold for reflex actions. Since the reflex effects of AC-stimulation surpassed the (A+C)-effects (Figs. 1-3) it was necessary to evaluate how much of the increase might have been due to the extra A-fibre activity. A typical example of this evaluation is shown in Fig. 4 in which the sympathetic reduction resulting from three series of A, C and AC-stimulations have been plotted together with the calculated influence of the extra A-fibre activity. A-stimulation was performed by heart synchronous trains of 80 Hz/80 ms duration 80/100 or 100/100 equivalent to respectively 6.8 and 10 pulses per heart beat. With a heart rate of 320 beats/min, the constant 2 Hz C-stimulation will hit less than two A trains per second, equal to an average increase of about 0.3 A-impulses per heart beat. From the recorded responses to A-stimulation alone (Fig. 4), this addition can be expected to produce a further 1.2% reduction in sympathetic activity. As shown in this figure and supported

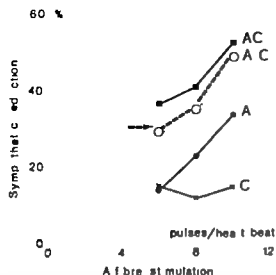


Fig. 4 Plot showing the calculated sympathetic effect of "extra" A fibre activity induced by the C stimulation of AC stimulations. Three stimulation series with different A fibre characteristics and a constant 1 Hz C-stimulation (abscissa valid only for A stimulations). Stimulation periods of 10 s each. See text for further description. In all three series the observed response to combined stimulation (AC) exceeded the sum of responses to separate A and C stimulations plus the calculated extra A-effect (arrow).

by similar observations in all other animals subjected to more than one type of A stimulation: the addition to (A+C)-effects of the calculated effects of the A fibre discharge caused by C stimulation was insufficient to explain the larger responses to AC than to (A+C)-stimulations.

Heart rate responses to stimulation were small always less than 10 beats/min and frequently absent. However in 23 series where at least one of the responses exceeded two beats/min: AC-effects were larger than (A+C) in all but two. Resting heart rates ranged from 230 to 320 beats/min.

The experiments allowed a comparison to be made between reflex responses to A and C-fibre stimulation. Relatively to the inhibition of renal nerve activity the effect on blood pressure was larger during A than C stimulation (Fig. 5).

DISCUSSION

In a study of interaction between central effects of electrically evoked activity in A and C-fibres of the same aortic nerve it is obviously essential that the afferent discharge induced by combined AC stimulation does not differ from the sum of separate A and C stimulations. This was not directly controlled

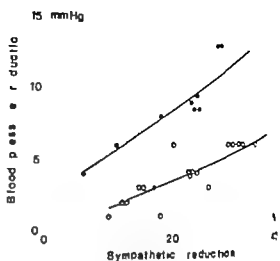


Fig. 5 A comparison between reductions in arterial blood pressure and sympathetic activity in the kidney during stimulation of aortic nerve C-fibres (open circles) at high frequencies and A-fibres at high frequencies (dots). Regression equations to the lines are respectively: $y = 0.18x - 0.2$, $r = 0.76$ and $y = 0.30x + 2.4$, $r = 0.65$. Same stimulation series as in Fig. 4.

In the present series, but the use of low-frequency stimulation of C-fibres makes it unlikely that collision between the simultaneously evoked action potentials in A fibres and the high-frequency potentials induced by the train stimulation of A-fibres should have much influenced the results. If anything, collision would have reduced the number of evoked potentials during combined- as compared to the sum of separate stimulation.

A more complex question is whether the excitation of A fibres by C stimulation, the extra A fibre activity exerted different central effects during AC than during C stimulations. The 2-4 Hz C stimulation equivalent to about 0.4-1 pulse/beat was undoubtedly below threshold for evoking reflex effects by action potentials in A-fibres (Fig. 4). However if during AC stimulation one or more of these A fibre activations coincided in time with the train stimulation of the same fibres, they presumably would add to the reflex effects of the other impulses in the train. There are two reasons for believing that the synergistic interaction demonstrated in Figs. 1-3 is not due to this type of artifact. First because the estimated effects of A fibre activity superimposed on the trains of A-fibre discharge invariably failed to explain the larger responses to AC than to (A+C)-stimulations (Fig. 4). Secondly because alterations in delay between start of A trains and C stimulation from 50 to 100 ms

did not affect the $AC(A+C)$ ratio. This was tried in one animal which unfortunately had no excluded from the material due to too severe spastic responses to stimulation (cf. Methods). The final prerequisite for the present study is that evoked afferent discharge did not exceed the μ of linearity of the reflex. To meet this requirement, I tentatively set the upper limits of responses at 60% reduction in renal nerve activity (25 mmHg drop in blood pressure (cf. Table)). While the linearity was not directly tested up to these values, the results in Figs. 2 and 3 as a predominance of inhibitory interactions as they occasionally were exceeded (excluded animals), probably constitute the best evidence that the limits were adequate for most animals.

Over the level of background sympathetic activity (i.e., activity not controlled by input from the aortic nerve) will vary from one animal to another. If particularly high, this background could partly mask the linear range of reflex control via the aortic nerve to less than 60% of renal nerve activity and hence make the response to combined stimulation smaller than it would have been in a normal system. For this reason, and because of the possibility of collision between A-fibre impulses elicited by the two pulse generators, I feel that the results employed in this study have underestimated rather than overestimated the synergistic relationship between activity in aortic nerve A- and C-fibres.

Results of the 'C on A'-stimulations cannot be compared to AC-stimulations due to variations in amount of reflex effects of the A-stimulation starting at the start of C stimulation. Time- and pressure-related influences from other baroreceptor areas are obviously important in this context, making the evaluation of these series more difficult than the AC vs. (A+C)-series. Comparison of responses to 'C on A' and C-stimulations did show, however, that the C-fibre discharge caused greater reflex changes in renal nerve activity and to some extent in arterial pressure, when the A-fibres were already activated. Thus, the synergistic interaction concerns central effects of discharge in A- and C-fibre afferent could be observed during two different modes of stimulation, as well as by two different parameters, arterial blood pressure and sympathetic activity to the kidney.

Previous investigations of reflex responses to specific stimulation of aortic nerve afferents have

mostly been limited to comparisons of effects of activity in myelinated vs. all fibres (Douglas, Ritchie & Schaumann 1956, Kardon, Peterson & Bishop 1973). Such studies have shown that activity in C-fibres will induce reductions in heart rate and blood pressure at discharge rates below threshold for elicitation of reflexes via A-fibres. Kardon, Peterson & Bishop (1975) also managed to study the effects of C vs. AC-stimulation by means of anodal block of the evoked A-fibre activity. Judged by the reflex changes in heart rate activity in A- and C-fibres was found to be about equipotent in the rabbits. It should be noted, however, that the fibres were stimulated at the same and rather high frequencies, far in excess of the discharge rate normally occurring in the C-fibres of the rabbit's aortic nerve (Thorén & Jones 1977). For this reason, and as their experiments were not designed to evaluate the effects of combined vs. separate stimulation of A- and C-fibres, their results are not relevant to the present series of experiments.

The question of summation of baroreceptor input from different nerves has repeatedly been studied but always without separation of effects via A- and C-fibres. In the most recent paper in this category Kendrick, Matson & Lalley (1979) reported that, in the dog, reflex changes in blood pressure, heart rate and vascular resistance in leg muscles in response to combined ipsilateral stimulation of the aortic and carotid sinus nerves exceeded the sum of separate stimulations. This illustration of synergistic interaction between reflexes from baroreceptor areas was performed by stimulation of mainly large afferents and the specific role of A- and C-fibres was left open in the discussion. The present investigation adds to our knowledge on this point by showing that synergistic interaction may exist also between reflex responses to activity in myelinated and non-myelinated fibres within the same baroreceptor nerve and that the reflex actions of activity in one type (C) can be potentiated by simultaneous activity in the other. The latter finding is highly relevant to the normal function of these fibres, since the pressure needed to activate receptors with C-fibres is well above the threshold for evoking A-fibre discharge in the same nerve.

Finally, it is worth noting that, relatively to the reflex changes in blood pressure, C-fibre activity evoked a stronger inhibition than A-fibre activity of the sympathetic discharge to the kidney (Fig. 5). Since heart rate changes were minimal (probably

due to anesthesia) the changes in blood pressure reflected the influence on sympathetic vasoconstrictor activity in general. Stronger effects of C than A fibre activity on renal nerve activity at equal pressure effects accordingly means that the reflex responses to aortic nerve A fibre activity must have surpassed the effects mediated by C fibre projections to sympathetic neurons serving other peripheral vascular beds. This deduction fits the observed responses to aortic nerve A and C fibre stimulation in cats relatively to blood pressure changes. A fibre activity produced a greater reduction in vascular resistance of calf muscle than did the C fibre activity (Öberg et al. personal communication). Taken together these observations illustrate interesting and important aspects of differentiated cardiovascular control.

This work was supported by The Norwegian Research Council for Science and the Humanities.

REFERENCES

- AARS H, MYHRE L. & HASWELL, H A 1978 The function of baroreceptor C fibres in the rabbit's aortic nerve. *Acta Physiol Scand* 102: 84-93.
 AKRE S & AARS H 1977 Pressure-independent inhibition of sympathetic activity by noradrenaline: role of baroreceptor C fibres. *Acta Physiol Scand* 100: 303-308.

- DOUGLAS W W, RITCHIE, J M. & SCHAMM, W 1956. Depressor reflexes from medullated and non-medullated fibres in the rabbit's aortic nerve. *J Physiol (Lond)* 132: 187-198.
 JONES J V & THORÉN P N 1977 Characteristics of aortic baroreceptors with non-medullated afferents arising from the aortic arch of rabbits with chronic renovascular hypertension. *Acta Physiol Scand* 101: 286-293.
 KARDON M B, PETERSON D F & BISHOP V S 1973 Reflex bradycardia due to aortic nerve stimulation in the rabbit. *Am J Physiol* 225: 7-11.
 KARDON M B, PETERSON D F & BISHOP V S 1975 Reflex heart rate control via specific aortic nerve afferents in the rabbit. *Circ Res* 37: 41-47.
 KENDRICK J E, MATSON G L & LALLEY P R 1979 Central interaction between the baroreceptor fibres from the carotid sinus and aortic arch. *Am J Physiol* 236: H127-H133.
 ÖBERG B, KENDRICK E, THORÉN P & WERNERGREN G 1980 Circulatory responses to stimulation of medullated and non-medullated afferents in the carotid sinus nerves and the aortic nerves in the cat. *To be published*.
 THORÉN P & JONES J V 1977 Characteristics of aortic baroreceptor C fibres in the rabbit. *Acta Physiol Scand* 99: 448-456.
 THORÉN P, SAUM W R. & BROWN A M 1977 Characteristics of aortic baroreceptors with non-medullated afferents in the rat. *Circ Res* 40: 231-237.

Evidence for differential localization of angiotensin I converting enzyme and renin in the corpus striatum of rat

ELL FUXE, DETLEV GANTEN, CHRISTER KÖHLER
AND SCHÜLL, and GEORG SPECK

Department of Histology, Karolinska Institute, Stockholm, Sweden, *Department of Pharmacology,
University of Heidelberg, West-Germany and Astra Laboratories, Södertälje, Sweden

Evidence for the presence of the components of a renin-angiotensin system in brain has been published (Ganten & Speck 1978, Phillips et al. 1979). In particular, the enzyme renin (E.C. 3.4.99.19) has been purified from brain tissue of several species including rat (Hirose et al. 1978), man (Ganten & Speck 1978) and mouse (Speck & Ganten in preparation). Brain renin was found to be distinct from plasma D-like acid proteases (Hirose et al. 1978, Ganten & Ganten in preparation, Osman et al. 1979, Anagnostou et al. 1978, Ganten & Speck 1978). The enzyme is immunologically similar to kidney and salivary gland renin.

The peptidyl dipeptide carboxylhydrolase (E.C. 3.4.15.1) which converts the decapeptide angiotensin-I (ANG I) to the effector octapeptide angiotensin-II (ANG II) has also been found to be present in brain (for review see Ganten & Speck 1978, Phillips et al. 1979). Angiotensin-II-like immunoreactivity has been demonstrated within certain types of nerve cells within the brain and spinal cord of the rat (Fuxe et al. 1976, Ganten et al. 1978). Specific angiotensin-II receptors seem to exist also in the mammalian brain (Bennett & Snyder 1976, Siret et al. 1977). Furthermore, a selective reduction of activity of angiotensin-I converting enzyme (E.C. 3.4.15.1) has been demonstrated in the corpus striatum of patients with Huntington's chorea (Arragon et al. 1977).

In the present paper evidence has been obtained of a neuronal location of angiotensin-I converting enzyme using ibotenic acid induced lesions of the corpus striatum (see Schwartz et al. 1979). Ibotenic acid, like kainic acid, produces a selective degeneration of the nerve cells within the injected area without damaging afferent nerve terminals. The local neurotoxic action of ibotenic acid does not

cause distant lesions in other parts of the brain as is the case with kainic acid (Schwartz et al. 1979).

Male specific pathogen-free Sprague Dawley rats were used (150-200 g b.w.). The right corpus striatum was lesioned by means of 4 stereotaxic injections with ibotenic acid as described by Fuxe et al. (1979). In some animals the extent of the lesion was studied by routine histological analysis using Nissl staining such as toluidine blue or thionine. The activity of angiotensin-I converting enzyme renin and cathepsin D-like acid protease was measured. The procedures and criteria of specificity for differential measurement of renin and cathepsin D-like acid protease have previously been published (Ganten & Speck 1978, Depierre & Roth 1975). For converting enzyme measurements N-carbobenzoyl-L-phenyl-alanine-L-histidine-L-leucine has been used.

Renin activity was expressed as picomoles angiotensin-I formed/mg protein/h. Cathepsin D-like acid protease activity was expressed in µg BSA equivalents/mg protein/h and specific angiotensin-I converting enzyme activity was expressed in picomoles of histidine-leucine/mg protein/min. The rats were killed 3 weeks after operation by rapid decapitation and the lesioned and unlesioned corpus striatum was dissected out and frozen for subsequent enzyme analysis.

The results are summarized in Table 1. The angiotensin-I converting enzyme activity is reduced by about 50% on the operated side compared with the unoperated side. However, the ibotenic acid induced degeneration of nerve cells in the corpus striatum produces an increase in renin and cathepsin D-like acid protease activity by about 50% on the lesioned side compared with the unoperated side (Table 1). A histological analysis showed an

due to anesthesia) the changes in blood pressure reflected the influence on sympathetic vasoconstrictor activity in general. Stronger effects of C than A fibre activity on renal nerve activity at equal pressure effects accordingly means that the reflex responses to aortic nerve A fibre activity must have surpassed the effects mediated by C fibre projections to sympathetic neurons serving other peripheral vascular beds. This deduction fits the observed responses to aortic nerve A and C fibre stimulation in cats relatively to blood pressure changes. A fibre activity produced a greater reduction in vascular resistance of calf muscle than did the C fibre activity (Öberg et al. personal communication). Taken together these observations illustrate interesting and important aspects of differentiated cardiovascular control.

This work was supported by The Norwegian Research Council for Science and the Humanities.

REFERENCES

- AARS H, MYHRE L. & HASWELL B A 1978 The function of baroreceptor C fibres in the rabbit's aortic nerve. *Acta Physiol Scand* 102: 84-93.
 AKRE S & AARS H 1977 Pressure-independent inhibition of sympathetic activity by noradrenaline: role of baroreceptor C fibres. *Acta Physiol Scand* 100: 303-308.

- DOUGLAS W W, RITCHIE J M & SCHAUMANN W 1956. Depressor reflexes from medullated and non-medullated fibres in the rabbit's aortic nerve. *Physiol (Lond)* 132: 187-198.
 JONES J V & THORÉN P N 1977 Characteristics of aortic baroreceptors with non-medullated afferents arising from the aortic arch of rabbits with chronic renovascular hypertension. *Acta Physiol Scand* 101: 286-293.
 KARDON M B, PETERSON D F & BISHOP V S 1973. Reflex bradycardia due to aortic nerve stimulation in the rabbit. *Am J Physiol* 225: 7-11.
 KARDON M B, PETERSON D F & BISHOP V S 1975. Reflex heart rate control via specific aortic nerve afferents in the rabbit. *Circ Res* 37: 41-47.
 KENDRICK J E, MATSON G L & LALLEY P J 1979. Central interaction between the baroreceptor reflexes from the carotid sinus and aortic arch in the rabbit. *Physiol* 236: H127-H133.
 ÖBERG U, KENDRICK E, THORÉN P & NERØREN G 1980. Circulatory responses to stimulation of medullated and non-medullated afferents in the carotid sinus nerves and the aortic nerve in the cat. (to be publ.)
 THORÉN P & JONES J V 1977. Characteristics of aortic baroreceptor C fibres in the rabbit. *Acta Physiol Scand* 99: 448-456.
 THORÉN P, SAUM W R. & BROWN A M 1977. Characteristics of aortic baroreceptors with non-medullated afferents in the rat. *Circ Res* 40: 231-237.

- DE, K., GANTEN D. HÖKFELT T. & BOLME, P. 1978. Immunohistochemical evidence for the existence of angiotensin II containing nerve terminals in the brain and spinal cord of the rat. *Neurosci Lett* 2: 229-234.
- GANTEN, D. & SPECK, G. 1978. The brain renin-angiotensin system: A model for the synthesis of peptides in the brain. *Biochem Pharmacol* 27: 2379-2389.
- GANTEN, D., FUXE, K., PHILLIPS, M. J., MANN J. F. E. & GANTEN U. 1978. The brain renin-angiotensin system: biochemistry, localization, and possible role in drinking and blood pressure regulation. In *Frontiers in neuroendocrinology* (ed. W. F. Ganong & L. Martini), pp. 61-99. Raven Press, New York.
- ROSE, S., YOKOSAWA H. & INAGAMI T. 1978. Immunohistochemical identification of renin in rat brain and distribution from acid proteases. *Nature* 274: 397-399.
- OSMAN M. Y., SENEBY R. R. & SEN S. 1979. Separation of dog brain renin-like activity from acid protease activity. *Hypertension* 1: 53-60.
- PHILLIPS M. J., WEYHENMEYER, J., FELIX, D., GANTEN D. & HOFFMAN W. E. 1979. Evidence for an endogenous brain renin-angiotensin system. *Fed Proc* 38: 2260-2266.
- SCHWARCZ, R., HÖKFELT T., FUXE, K., JONSSON O., GOLDSTEIN M. & TERENIUS, L. 1979. Ibotenic acid-induced neuronal degeneration. A morphological and histochemical study. *Exp Brain Res* 37: 199-216.
- SIRETT N. E., MCLEAN A. S., BRAY J. J. & HUBBARD J. I. 1977. Distribution of angiotensin II receptors in rat brain. *Brain Res* 122: 299-312.
- SPECK, G. & GANTEN D. 1980. In preparation.

Table 1 The effects of ibotenic acid induced lesions of the corpus striatum of normal male rats on angiotensin I converting enzyme activity, renin enzyme activity and cathepsin D-like acid protease activity. For details on the operation procedure see text. Means \pm S.E. are shown out of 10-11 rats. Student's *t*-test was used for the statistical analysis.

	Lesioned striatum	Statistical difference Lesioned vs. Intact striatum	Unlesioned striatum
Converting enzyme (pmol his-leu/mg protein/min)	35 \pm 38		744 \pm 40
Renin (pmol ANG I/mg protein/h)	12 \pm 1 ⁹		67 \pm 10
Cathepsin D-like acid protease (μ g BSA/mg protein/h)	1496 \pm 397		726 \pm 44

$p < 0.01$ $p < 0.005$

almost complete degeneration of nerve cells within the corpus striatum of the lesioned rats analyzed.

Ibotenic acid produces a selective neuronal degeneration within the corpus striatum without damaging glial elements and extrinsic nerve terminals and axons of passage (Schwarcz et al 1979). The present findings therefore give evidence that the angiotensin-I converting enzyme has a neuronal localization within the corpus striatum. These findings are in agreement with the depletion of this enzyme found in the corpus striatum of patients with Huntington's chorea (Arregui et al 1977). The discovery that renin and cathepsin D-like acid protease were increased about 50% following neuronal degeneration of the striatum warrants further methodological and functional investigations. It indicates that both enzymes are stored in another cellular compartment than angiotensin I converting enzyme. One possibility is that both enzymes are located within glial elements of the corpus striatum since these elements may proliferate following ibotenic acid induced degeneration of the nerve cells. Another possibility is that renin is located within the extrinsic nerve terminals of the corpus striatum since these terminals remain intact following ibotenic acid induced neuronal degeneration. The increase in renin enzyme activity could be looked upon as a compensatory increase due to reduced amounts of angiotensin II present within the corpus striatum in view of the reduction of angiotensin-I converting enzyme activity. Renin enzyme activity has in fact been demonstrated within synaptosomes (see Ganten et al 1978). This problem concerning the localization of renin will be solved by the use of a proper immunohistochemical analysis of the localization of renin like immunoreactivity. It should also be noticed that 50%

of the angiotensin-I converting enzyme remains within the corpus striatum which completely lacks nerve cells. Thus angiotensin-I converting enzyme is located partly within striatal nerve cells and partly within extrinsic striatal nerve terminals and within glial elements.

In conclusion 50% of the angiotensin-I converting enzyme is located within striatal nerve cells which probably do not contain appreciable amounts of renin indicating that at least in some nerve cells renin and angiotensin I converting enzyme may be differentially stored.

This work has been supported by a grant (04X 715) from the Swedish Medical Research Council by a grant from Magnus Bergvall's Stiftelse, Karolinska Institute, Stockholm, and by the Deutsche Forschungsgemeinschaft (DFG) within the SFB90 Cardiovasculäres System.

REFERENCES

- ARREGUI A, BENNETT J P, BIRD E D, YAMAMURA H I, IVERSEN L L & SNYDER S H 1977 Huntington's chorea. Selective depletion of activity of angiotensin converting enzyme in the corpus striatum. *Ann Neurol* 2: 294-298.
- BENNETT J B & SNYDER S H 1976. Angiotensin binding to mammalian brain membranes. *J Biol Chem* 251: 7423-7430.
- DEPIERRE D & ROTH M 1975 Fluorimetric determination of dipeptidyl carboxypeptidase (angiotensin converting enzyme). *Enzyme* 19: 65-70.
- DWARSCHACK T, GREGORY T J & PRINTZ L P 1978 Identification of neutral pH renin-like activity from brain homogenates by chromatography on phenylethylamine sepharose. *Fed Proc* 38: 1305.
- FUXE K, HALL H & KÖHLER C 1979 Evidence for an exclusive localization of ¹²⁵I-ADTN binding sites to postsynaptic nerve cells in the striatum of the rat. *Eur J Pharmacol* 58: 515-517.

Experimental evidence for neurotensin as a metabolite being a hormone

ROSELL

Department of Pharmacology, Karolinska Institute, Stockholm, Sweden

Neurotensin, a tridecapeptide, is stored in specific enteroendocrine cells (N-cells) in the mucosa of the ileum and jejunum and, to a lesser extent, in the colon and duodenum (Carraway & Leeman 1976; Helander et al 1977). Folkers et al (1976) suggested that (Gln)-neurotensin rather than neurotensin may be a naturally occurring peptide. In dogs higher concentrations of neurotensin-like immunoreactivity (NTLI) are found in blood draining from the ileum than in arterial blood (Mashford et al 1978) indicating that neurotensin is released into the blood on the onset of a meal and particularly fat. In rats a major influence on plasma NTLI. Ingestion of fat (35 ml triolein 20%) increases the plasma NTLI concentration from 52 ± 7 pM to 109 ± 77 pM. 100 g berries isocaloric amounts of amino acids and glucose have negligible effects (Rosell & Åkesson 1979).

In small doses administered i.v. to dogs neurotensin or (Gln)-neurotensin inhibits gastric acidity (Andersson et al 1977) and suppresses gastric acid secretion (Andersson et al 1976). It also increases blood flow in the gastrointestinal region and decreases it in subcutaneous adipose tissue (Rosell et al 1976). In man i.v. infusion of (Gln)-neurotensin or (Gln)-neurotensin inhibits gastric acidity, the lower esophageal sphincter (LES) pressure from 14 mmHg to 5 mmHg with return to preinfusion pressure within 15 min after cessation of 5 min infusion period. The decrease occurs at plasma NTLI concentrations below those obtained in man after a meal or ingestion of fat (Rosell et al 1980).

The experimental data show that neurotensin or a metabolite, satisfies rapid physiological criteria that should be met before a substance is classified as a hormone with endocrine functions. Thus the release of NTLI into the blood is produced by a physiologically meaningful event (ingestion of food especially fat) and gastrointestinal actions following infusion of (Gln)-neurotensin are produced at lower plasma NTLI concentrations than those obtained following ingestion of food. Neurotensin or a

metabolite may function as an endocrine hormone involved in the postprandial regulation of the gastric acid secretion and the motility of the gastrointestinal tract. Thus neurotensin may function as an enterogastrose. In addition neurotensin may regulate the postprandial distribution of fat by adjusting the adipose tissue blood flow.

REFERENCES

ANDERSSON S, CHANG D, POLKERS K & ROSELL, S. 1976. Inhibition of gastric acid secretion in dogs by neurotensin. *Life Sci* 19: 367-370.
ANDERSSON S, ROSELL, S., HJELMQVIST U., CHANG D & POLKERS K. 1977. Inhibition of gastric and intestinal motor activity in dogs by (Gln)-neurotensin. *Acta Physiol Scand* 100: 231-235.
CARRAWAY R E & LEEMAN S E. 1976. Characterization of radioimmuno-assayable peptide neurotensin in the rat; its differential distribution in the central nervous system, small intestine and stomach. *J Biol Chem* 251: 7045-7052.
POLKERS, K, CHANG, D, HUMPHRIES, J, CARRAWAY R., LEEMAN S E. & BOWERS, C. J. 1976. Synthesis and activities of neurotensin and its acid and amide analogues. Possible natural occurrence of (Gln)-neurotensin. *Proc Natl Acad Sci (USA)* 73: 3833-3837.
HELMSTAEDTER, V, TAUGNER, C H, FEURLE, G. E. & FORSMANN W G. 1977. Localization of neurotensin immunoreactive cells in the small intestine of man and various mammals. *Histochemistry* 53: 35-41.
MASHFORD W L, NILSSON O, RÖKAEUS Å. & ROSELL, S. 1978. Release of neurotensin-like immunoreactivity (NTLI) from the gut in anaesthetized dogs. *Acta Physiol Scand* 104: 375-376.
ROSELL, S, BURCHER, E, CHANG, D & POLKERS, K. 1976. Cardiovascular and metabolic actions of neurotensin and (Gln)-neurotensin. *Acta Physiol Scand* 96: 484-491.
ROSELL, S. & RÖKAEUS Å. 1979. The effect of ingestion of amino acids, glucose and fat on circulating neurotensin-like immunoreactivity (NTLI) in man. *Acta Physiol Scand* 107: 263-267.
ROSELL, S, THOR, K, RÖKAEUS, Å., NYQVIST O, LEWENHAUPT A, KAUER, L. & POLKERS, K. 1980. Plasma concentration of neurotensin-like immunoreactivity (NTLI) and lower esophageal sphincter (LES) pressure in man following infusion of (Gln)-neurotensin. *Acta Physiol Scand* 109: 369-375.

[¹²⁵I]-Neurotensin and gastrointestinal motility in man

THOR. Å. RÖRÆUS, L. KAGER² and S. ROSELL

From the ¹Physiol Clinic, Ersta Hospital, ²Department of Pharmacology ³Department of Surgery Huddinge Hospital and ⁴Enviromental Institute, Stockholm, Sweden

In the upper digestive tract decreases gastric motor activity (E. Ald & Boas 1886). The inhibition has been suggested to be caused by a hormone from the intestinal mucosa which was named enterogastric (Kosaka & Lim 1930). Several hormones including gastrin, secretin, cholecystokinin and somatostatin are released after food intake and have been ascribed to have a role as enterogastric. However none of them has been shown to be the possible hormone. Neurotensin has pronounced effects on motility in the gastrointestinal tract of the rat (Andersson et al 1977) and in man the plasma NTLI increase following ingestion of fat (Rosell & Årner 1979). Neurotensin is therefore an enterogastric candidate.

In order to see if intravenous infusions of [¹²⁵I]-neurotensin affect gastrointestinal motor activity in man the pressures at antrum, proximal and distal duodenum, respectively were measured in six healthy male volunteers. The pressures were measured with three water-filled constantly perfused catheters (0.08 ml min⁻¹) each having a lateral orifice 10 cm apart. [¹²⁵I]-neurotensin was infused intravenously (6 pmol kg⁻¹ min⁻¹) during 20 min.

During infusion of [¹²⁵I]-neurotensin the peristaltic activity (pressure waves above 5 mmHg in antrum progressing to one or two of the recording catheters in the duodenum) was completely abolished ($P < 0.001$) from a resting value of 3.4 ± 0.6 contractions $\times 5$ min⁻¹. After cessation of the infusion activity returned to preinfusion levels. Moreover the number of antral contractions above 5 mmHg but without any progression to duodenum was reduced but not totally abolished. The inhibition occurred at plasma NTLI concentrations below those obtained in man after a meal (Mashford et al 1978).

In the fasting state regular motor activity consisting of bursts of contractions migrating caudally and returning cyclically may be recorded in the small intestine of several species including man (Van-

trappen et al 1977). After ingestion of food and especially after ingestion of fat these motor complexes are abolished and the motor activity changes into the 'fed' type.

In 6 healthy male volunteers the pressures were measured in the proximal duodenum, in the flexura duodenojejunalis and in the jejunum 5 cm distal to the angle of Treitz. Three water-filled constantly perfused catheters (0.08 ml min⁻¹) were used each having a lateral orifice 5 cm apart. [¹²⁵I]-neurotensin was infused i.v. (6 pmol kg⁻¹ min⁻¹) during 200 min.

The intervals between the interdigestive migrating motor complexes were significantly ($P < 0.001$) prolonged from 111 ± 14 min before infusion to 226 ± 8 min during the infusion period. The motility pattern changed into the fed type during the infusion period. The inhibition of the interdigestive migrating motor complexes also occurred at plasma NTLI levels below those obtained after a meal.

[¹²⁵I]-neurotensin inhibits the peristaltic activity of the stomach and duodenum and the interdigestive migrating motor complexes in the small intestine in man. The present data support the hypothesis that in man plasma neurotensin or a neurotensin metabolite is an endocrine hormone involved in the postprandial regulation of the motor functions of the gastrointestinal tract.

REFERENCES

- ANDERSSON S, ROSELL S, HJELMQUIST U, CHANG D & FOLKERS, K. 1977 Inhibition of gastric and intestinal motor activity in dogs by [¹²⁵I]-neurotensin. *Acta Physiol Scand* 100: 231-235.
- EWALD C. A. & BOAS, J. 1886 Beiträge zur Physiologie und Pathologie der Verdauung. *Virchows Arch* 104: 271-305.
- KOSAKA, T & LIM R. R. S. 1930 Demonstration of the humoral agent in fat inhibition of gastric secretion. *Proc Soc Exp Biol NY* 27: 890-891.

Release of neurotensin like immunoreactivity (NTLI) from rat intestine following administration of bombesin

Å RÖKAEUS and T McDONALD

Department of Pharmacology Karolinska Institutet Stockholm Sweden and Department of Medicine University Hospital London Ontario Canada

In man fat ingestion causes a pronounced increase in plasma NTLI presumably due to a direct exposure of the gastrointestinal tract to nutrients but possibly also by the action of some releasing hormone (Rosell & Rökæus 1979). Bombesin a tetradecapeptide isolated from frog skin (Anastasi et al 1971) has been reported to increase plasma neurotensin levels upon administration to man (Lezoché et al 1979). Recently a porcine gastric heptacosapeptide having striking C terminal homology with bombesin has been isolated (McDonald et al 1979).

The purpose of this investigation was to quantify rat plasma NTLI increases following parenteral administration of bombesin to attempt localization of the site of NTLI release and to investigate the possibility that a bombesin like substance may be implicated in fat induced elevation of plasma NTLI.

In conscious rats tail vein injections of bombesin (7.8 to 250 pmol/kg) resulted in dose dependent plasma NTLI increases the maximum increase being obtained with 125 pmol/kg. Injection of 125 pmol/kg increased plasma NTLI to 139 ± 6.4 pM (mean \pm S.E.) 78 ± 4 , 73 ± 5 , 73 ± 3.4 and 55 ± 3.7 pM at 1, 3, 5, 10 and 20 min respectively ($n=6$). All values were significantly elevated as compared to a saline control value of 38 ± 1.6 pM at 3 min.

Anesthetized rats ($n=4$) were given 70 min femoral vein infusions of bombesin at doses of 3.1 and 31.7 pmol/kg/min respectively. An initial peak of plasma NTLI at 3 min was followed by a second gradual rise lasting throughout the remainder of the infusion.

Bombesin (125 pmol/kg⁻¹) when injected into the aorta at the level of the celiac artery the superior mesenteric artery or just distal to the superior mesenteric artery with the two other vascular branches

clamped ($n=5$) resulted in significant increases in plasma NTLI only after injections into the superior mesenteric artery a maximal value of 102 ± 11 pM (basal 77 ± 3.6) was seen at 45 s.

In anesthetized rats intragastric fat administration (7.5 ml Intralipid 20%) increased plasma NTLI from a basal value of 22 ± 1 pM to 6.1 ± 1.6 , 5.5 ± 0.9 at 70 and 60 min respectively ($n=5$). However no significant increase of plasma NTLI was seen with intragastric fat when a ligature was placed around the duodenum 1–2 proximal to the ligament of Treitz ($n=3$).

The findings suggest that bombesin releases NTLI in a dose related fashion from the intestinal region perfused from the superior mesenteric artery (area corresponding to the location of most of the N-cells). However a bombesin-like substance located in the proximal gastrointestinal tract is unlikely to be a mediator of Intralipid induced plasma NTLI increase in the rat.

REFERENCES

- ANASTASI A, ERSPANER V & BUCCI M 1971 Isolation and structure of bombesin and other related analogous active peptides from the skin of the European amphibians Bombina and Alytes. *Experientia* 27: 166–167.
- LEZOCHÉ E, GHATEI M A, CARLÉ J, BLACKBURN A M, BASSO N, ADRIAN T, SPERANZA V & BLOOM S R 1979 Gut hormone response to bombesin in man. *Gastroenterology* 76: 1185.
- McDONALD T J, JÖRNVALL H, NILSSON L, VAGNE M, GHATEI M, BLOOM S R, MUTT V 1979 Characterization of a gastric releasing peptide from non-antral gastric tissue. *Biochem Biophys Res Comm* 90: 227–233.
- ROSELL S & RÖKAEUS Å 1979 The effect of ingestion of amino acids, glucose and fat on circulating neurotensin-like immunoreactivity (NTLI) in man. *Acta Physiol Scand* 107: 2.

[Gln]-Neurotensin and gastrointestinal motility in man

THOR, Å. RÖKAEUS, L. KAGER² and S. ROSELL

From the Dept. of Surgery, Ersta Hospital, ¹Department of Pharmacology, ²Department of Surgery, Huddinge Hospital, Huddinge, Sweden

In the upper digestive tract decreases gastric motor activity (Ewald & Boas 1886). The inhibition has been suggested to be caused by a hormone from the small intestine which was named enterogastrone (Kosaka & Lim 1930). Several hormones including gastrin, secretin, cholecystokinin and somatostatin are released after food intake and have been suggested to have a role as enterogastrone. However none of them has been shown to be the responsible hormone. Neurotensin has pronounced effects on motility in the gastrointestinal tract of the dog (Andersson et al. 1977) and in man the plasma NTLI increase following ingestion of fat (Rosell & Skerfving 1979). Neurotensin is therefore an enterogastrone candidate.

In order to see if intravenous infusions of [Gln]-neurotensin affect gastrointestinal motor activity in man the pressures in antrum, proximal and distal duodenum, respectively were measured in six healthy male volunteers. The pressures were measured in three water-filled constantly perfused catheters (0.08 ml/min), each having a lateral orifice 10 cm apart. [Gln]-neurotensin was infused intravenously (6 pmol/kg/min) during 20 min.

During infusion of [Gln]-neurotensin the peristaltic activity (pressure waves above 5 mmHg in antrum progressing to one or two of the recording catheters in the duodenum) was completely abolished ($P < 0.001$) from a resting value of 3.4 ± 0.6 contractions/min. After cessation of the infusion activity returned to preinfusion levels. Moreover the number of antral contractions above 5 mmHg but without any progression to duodenum was reduced but not totally abolished. The inhibition occurred at plasma NTLI concentrations below those obtained in man after a meal (Mashford et al. 1978).

In the fasting state regular motor activity consisting of bursts of contractions migrating caudally and recurring cyclically may be recorded in the small intestine of several species including man (Van-

trappen et al. 1977). After ingestion of food and especially after ingestion of fat these motor complexes are abolished and the motor activity changes into the 'fed' type.

In 6 healthy male volunteers the pressures were measured in the proximal duodenum at the flexura duodenojejunalis and in the jejunum 25 cm distal to the angle of Treitz. Three water-filled constantly perfused catheters (0.08 ml/min) were used each having a lateral orifice 25 cm apart. [Gln]-neurotensin was infused (6 pmol/kg/min) during 20 min.

The intervals between the interdigestive migrating motor complexes were significantly ($P < 0.001$) prolonged from 111 ± 14 min before infusion to 226 ± 8 min during the infusion period. The motility pattern changed into the fed type during the infusion period. The inhibition of the interdigestive migrating motor complexes also occurred at plasma NTLI levels below those obtained after a meal.

[Gln]-neurotensin inhibits the peristaltic activity of the stomach and duodenum and the interdigestive migrating motor complexes in the small intestine in man. The present data support the hypothesis that in man plasma neurotensin or a neurotensin metabolite is an endocrine hormone involved in the postprandial regulation of the motor functions of the gastrointestinal tract.

REFERENCES

- ANDERSSON S., ROSELL S. & HJELMQUIST U. CHANG D. & FOLPERS K. 1977. Inhibition of gastric and intestinal motor activity in dogs by [Gln]-neurotensin. *Acta Physiol Scand* 100: 231-235.
- EWALD, C. A. & BOAS J. 1886. Beiträge zur Physiologie und Pathologie der Verdauung. *Virchows Arch* 164: 771-305.
- KOSAKA, T. & LIM R. R. S. 1930. Demonstration of the humoral agent in fat inhibition of gastric secretion. *Proc Soc Exp Biol NY* 27: 890-891.

- MASUOYORI M I, NILSSON G, RÖKAIUS Å & ROSH L S 1978 The effect of food ingestion on circulating neurotensin-like immunoreactivity (NII) in the human. *Acta Physiol Scand* 104: 44-46.
- ROSH L S & RÖKAIUS Å 1979 The effects of ingestion of amino acid, glucose and fat on circulating neurotensin-like immunoreactivity (NII) in man. *Acta Physiol Scand* 107: 63-67.
- VANTRAPPEN G, JANSSENS J, HILLMANST, CHRISTOFFIDIS N & BLOOM S 1978 Effect on the interdigestive (migrating) motor complex in man. *Proceeding of the Sixth International Symposium on Gastrointestinal Motility* (1977) Ed H L Roth. MTP Press Ltd, Lancaster.

Inhibition of pentagastrin stimulated gastric acid secretion and rise in the plasma concentration of neurotensin like immunoreactivity (NTLI) by intraduodenal oleic acid in man

KIHL, Å RÖKAEUS* & ROSELL and L. OLBE

Department of Surgery II, University of Göteborg, Sweden, and *Department of Pharmacology, Karolinska Institute, Stockholm, Sweden

The effects of intraduodenal administration of oleic acid (5, 10, 20 and 40 ml) on gastric acid secretion and plasma concentration of neurotensin-like immunoreactivity (NTLI) were studied in 18 healthy subjects. Gastric acid secretion was stimulated by a standard L-pentagastrin infusion, the amount of which was determined on the basis of individual pentagastrin dose-response tests. We also studied 6 duodenal ulcer patients before and after proximal gastric vagotomy. Gastric acid secretion and plasma NTLI were followed during a 120 min period after the intraduodenal instillation of oleic acid.

5 ml oleic acid administered intraduodenally significantly inhibited gastric acid secretion by 29% and there was a significant rise in plasma NTLI with peak concentration at 30 min. Maximal inhibition of acid secretion occurred after 20 ml oleic acid (41%). Over the 120 min period there was a close temporal relation between the inhibition of gastric acid secretion and the rise in plasma NTLI. In addition, the correlation between individual inhibition of gastric acid secretion and the individual integrated plasma NTLI response to oleic acid was statistically significant. These results together with other data (Andersson et al 1976) suggest that neurotensin or a metabolite may account for the suppression of gastric acid secretion caused by oleic acid. This is in accordance with the hypothesis that neurotensin may have an enterogastrone-like function (Rosell & Rökæus 1979).

In the duodenal ulcer patients 20 ml of oleic acid evoked a 70% inhibition of gastric acid secretion which was significantly lower when compared to that obtained in the healthy group. Moreover the peak plasma NTLI concentrations were significantly lower in the duodenal ulcer patients than in the healthy group. Proximal gastric vagotomy abolished the inhibition of gastric acid secretion by oleic acid. The total integrated plasma NTLI response was the same before and after proximal gastric vagotomy. These findings confirm earlier results that neurotensin needs an intact vagal innervation to suppress gastric acid secretion (Andersson et al 1980). Moreover the data indicate that duodenal ulcer patients may have a defective fat inhibitory mechanism.

REFERENCES

- ANDERSSON S, ROSELL S, SJÖDIN L. & FOLKERS K. 1980 Inhibition of acid secretion from vagally innervated and denervated gastric pouches by (Gln⁶)-neurotensin. *Scand J Gastroenterol* 15: 253-256.
- ANDERSSON S, CHANG D., FOLKERS K. & ROSELL S. 1976 Inhibition of gastric acid secretion in dogs by neurotensin. *Life Sciences* 19: 367-370.
- ROSELL S & RÖKAEUS, Å. 1979 The effect of ingestion of amino acids, glucose and fat on circulating neurotensin-like immunoreactivity (NTLI) in man. *Acta Physiol Scand* 107: 263-267.

- MASHFORD M L, NILSSON G, RÖKAEUS Å & ROSELL, S. 1978 The effect of food ingestion on circulating neurotensin like immunoreactivity (NTLI) in the human. *Acta Physiol Scand* 104: 44–46.
- ROSELL, S & RÖKAEUS Å. 1979 The effect of ingestion of amino acids, glucose and fat on circulating neurotensin-like immunoreactivity (NTLI) in man. *Acta Physiol Scand* 107: 263–267.
- VANTRAPPEN G, JANSSENS, J, HELLEMAN, J & CHRISTOFIDES N & BLOOM, S. 1978 Effect on the interdigestive (migrating) motor complex in man. *Proceeding of the Sixth International Symposium on Gastrointestinal Motility* (1977). Ed. H. L. Doty. MTP Press Ltd, Lancaster.

Inhibition of pentagastrin stimulated gastric acid secretion and rise in the plasma concentration of neurotensin like immunoreactivity (NTLI) by intraduodenal oleic acid in man

KHIL, Å RÖKAEUS, S. ROSELL and L. OLBE

Department of Surgery II, University of Göteborg, Sweden, and ²Department of Pharmacology, Karolinska Institute, Stockholm, Sweden

Effects of intraduodenal administration of oleic acid (5, 10, 20 and 40 ml) on gastric acid secretion and plasma concentration of neurotensin-like immunoreactivity (NTLI) were studied in 18 healthy subjects. Gastric acid secretion was stimulated by a biperidol-L-pentagastrin infusion, the amount of acid secreted was determined on the basis of individual pentagastrin dose-response tests. We also studied 6 duodenal ulcer patients before and after proximal gastric vagotomy. Gastric acid secretion and plasma NTLI are followed during a 120 min period after the intraduodenal instillation of oleic acid.

5 ml oleic acid administered intraduodenally significantly inhibited gastric acid secretion by 29% and there was a significant rise in plasma NTLI with peak concentration at 30 min. Maximal inhibition of acid secretion occurred after 20 ml oleic acid (77%). Over the 120 min period there was a close temporal relation between the inhibition of gastric acid secretion and the rise in plasma NTLI. In addition, the correlation between individual inhibition of gastric acid secretion and the individual integrated plasma NTLI response to oleic acid was statistically significant. These results together with other data (Andersson et al 1976) suggest that neurotensin or a metabolite may account for the inhibition of gastric acid secretion caused by oleic acid. This is in accordance with the hypothesis that neurotensin may have an enterogastrone-like function (Rosell & Rökæus 1979).

In the duodenal ulcer patients 20 ml of oleic acid evoked a 20% inhibition of gastric acid secretion which was significantly lower when compared to that obtained in the healthy group. Moreover the peak plasma NTLI concentrations were significantly lower in the duodenal ulcer patients than in the healthy group. Proximal gastric vagotomy abolished the inhibition of gastric acid secretion by oleic acid. The total integrated plasma NTLI response was the same before and after proximal gastric vagotomy. These findings confirm earlier results that neurotensin needs an intact vagal innervation to suppress gastric acid secretion (Andersson et al 1980). Moreover the data indicate that duodenal ulcer patients may have a defective fat inhibitory mechanism.

REFERENCES

- ANDERSSON S, ROSELL S, SJÖDIN L. & FOLKERS K. 1980 Inhibition of acid secretion from vagally innervated and denervated gastric pouches by (Gln¹)-neurotensin. *Scand J Gastroenterol* 11: 253-256.
- ANDERSSON S, CHANG B, FOLKERS K. & ROSELL S. 1976. Inhibition of gastric acid secretion in dogs by neurotensin. *Life Sciences* 19: 367-370.
- ROSELL S. & RÖKAEUS, Å. 1979. The effect of ingestion of amino acids, glucose and fat on circulating neurotensin-like immunoreactivity (NTLI) in man. *Acta Physiol Scand* 107: 263-267.

Plasma NTLI after administration of fat to obese volunteers and patients operated with jejunio ileal bypass

B. WIKLUND¹, Å. RÖKAEUS², D. HALLBERG¹ and S. ROSELL²

¹Department of Surgery, Huddinge sjukhus and ²Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden

The aim of the present study was to compare the plasma levels of neurotensin like immunoreactivity (NTLI) after the oral administration of fat between healthy and obese volunteers and patients operated for obesity with jejunio-ileal bypass, where a large proportion of the N-cell containing small intestine is shunted away from the contact with food.

Plasma NTLI was determined after the oral administration of 55 ml 70% Intralipid (11 g of fat mainly as triglycerides) to 3 groups of subjects:

(a) 6 healthy volunteers weighing on the average 62 kg

(b) 6 very obese but otherwise healthy volunteers weighing on the average 137 kg

(c) 70 patients who 10 years previously had been operated with jejunio-ileal bypass and there after decreased their average weight from 138 to 87 kg

After fat administration to the healthy volunteers the plasma concentration of NTLI increased significantly from 43 ± 5 pM to 85 ± 13 pM at 30 min. In the obese volunteers fat caused an increase of NTLI concentration from 77 ± 6 pM to a maximum of 50 ± 10 pM at 90 min. At 30 and 60 min after the fat administration the NTLI concentrations were significantly ($P < 0.01$) lower than for non-obese volunteers. In the patients with jejunio-ileal bypass the NTLI concentration increased from 34 ± 4 pM to a maximum of 125 ± 19 pM at 180 min which was significantly ($P < 0.01$) higher than in the obese volunteers.

These data indicate that plasma NTLI levels are

lower in very obese volunteers than in healthy ones. Fat induced increases in plasma NTLI were similar although delayed in obese subjects. Moreover 10 years after jejunio-ileal bypass the NTLI response was larger than in non-operated obese subjects. The reasons for these differences are not clear. The number of N-cells exposed to ingested fat may have been quite different in the obese and normal subjects. After jejunio-ileal bypass the number of N-cells may have increased in the functioning intestine which is well known to hypertrophy (Polak et al. 1978; Besterman et al. 1978). Alternatively the dose response curve may have been displaced to the left with increased sensitivity of the N-cell to fat. Another possibility is that some yet unknown substance released from the proximal intestine may cause the increased response in plasma NTLI.

REFERENCES

- POLAK J. M., HOBBS S. E., BLOOM S. R., BESTERMAN H. S., GREENBERG H. R., PEARSON A. G. E., CLEARY J., GAZET J. C., MONSON J. & PILKINGTON R. E. 1978. Neurotensin and teroglucaagon in obesity. *Gastroenterology* 74: 1078.
- BESTERMAN H. S., SARSON D. L., BLACKBURN A. M., CLEARY J., PILKINGTON T. R. E., BLOOM S. R. 1978. The gut hormone profile in adult obesity and following jejunio-ileal bypass. *Scandinavian Journal of Gastroenterology*. The 7th International Symposium on Gastrointestinal Hormones (ed. J. Myren, L. Schrampe, L. E. Hanssen & M. Vatn), Vol. 13, Sept. 49, p. 15.

Biological profile of leukotrienes C₄ and D₄

R. HEDQVIST, SVEN ERIK DAHLÉN, LARS GUSTAFSSON
EN HAMMARSTRÖM and BENGT SAMUELSSON

Department of Physiology and Chemistry, Karolinska Institute, Stockholm, Sweden

ACTA

A reacting substance of anaphylaxis (SRS-A) has long been recognized as a smooth muscle active agent or family of compounds. As presumed mediator of anaphylactic reactions it has been mainly linked to perturbations of lung mechanics (Orange & Ammon 1969). It was recently found that SRS-A (Murphy et al. 1979) belongs to a novel group of compounds, the leukotrienes (Borgeat & Smith 1979). These are formed from arachidonic acid by a pathway initially involving oxidation of 5-hydroperoxy derivative (Fig. 1). The chemical structures of SRS-A from various sources have recently been determined and the metabolic pathways involved in their formation and other transformations have been elucidated as indicated in Fig. 1 (Samuelsson et al. 1980).

In this communication we wish to report on some biological effects of leukotrienes C and D (LTC₄, LTD₄), with special reference to previous information on biological activities of crude SRS-A. These are presented at the 1980 Prostaglandin Interim Conference in Snowbird, Utah, USA.

In following test systems were used, helical strips of guinea pig bronchi and guinea pig trachea, strips from guinea pig paracystic, ileum (longitudinal muscle layer only or longitudinal segments) and uterine horns (virgin or ovariectomized 4 weeks before the experiment), tracheal insufflation pressure and dermal vascular permeability in guinea pigs (all systems sensitive to histamine), and the relatively SRS-A insensitive rabbit intestinal and bronchial strips, and strips from rat uterus. Strip tests were performed at 37°C in 5 ml baths containing Tyrode or de Jalon (uterine horn) solutions bubbled with 5% CO₂ in O₂. Contractions were recorded mechanically (Grass FT 83) or isotonically (subintestinal and uterus strips, Harvard J46, load 0.25-0.5 g).

Studies on insufflation pressure and vascular permeability are done according to Strandberg & Hedqvist (1975) and Orange & Ammon (1968) respectively.

LTC₄ and LTD₄ (0.1-10 nM) caused concentration-dependent contractions of guinea pig ileum, but they did not contract rabbit duodenal strips in

concentrations up to 40 nM. The guinea pig ileum is interesting from the point of view that it is a classical SRS-A assay preparation widely used to define the biological activity of one unit SRS-A in relation to that of histamine. In the present study histamine was on a molar basis 700 times less active than LTC₄ suggesting that one unit of SRS-A corresponds to approximately 0.2 pmol LTC₄.

Likewise LTC₄ dose-dependently contracted uterine strips from virgin (0.1-10 nM) and adult ovariectomized (10-100 nM) guinea pigs being approximately 300 times more potent than histamine in the two preparations. Uterine horns from rats brought into artificial estrus proved insensitive to LTC₄ (100 nM) and histamine (10 µM) but they contracted vigorously to 100 nM PGE₂.

LTC₄ and LTD₄ were remarkably potent and largely equiactive in causing increased vascular permeability in guinea pig skin. Thus, 0.1 pmol (0.1 ml) injected intradermally was sufficient to produce (in 30 min) blue spots (due to leakage of circulating Evans blue) of significantly greater intensity and size than those caused by NaCl (solvent) or histamine (0.1 pmol).

Pulmonary effects of leukotrienes are of particular interest, considering that SRS-A may have a mediator role in anaphylactic reactions. Intravenous injection (0.2 ml into jugular vein) of LTC₄ and histamine caused dose-related and parallel increases in insufflation pressure in anesthetized and artificially ventilated guinea pigs (Fig. 1). LTD₄ was virtually equipotent with LTC₄, the ED₅₀ (the dose that causes 50% increase in insufflation pressure) being 89 and 120 pmol respectively and they were more than 100 times more potent than histamine (ED₅₀ = 14 nmol). The leukotrienes were at least as potent when they were given as aerosols indicating that the increase in insufflation pressure was due to bronchoconstriction. Notably the results are in excellent harmony with previous information on effects of SRS-A in this test system (Strandberg & Hedqvist 1975).

Plasma NTLI after administration of fat to obese volunteers and patients operated with jejuno ileal bypass

B WIKLUND¹, Å RÖKAUS², D HALLBERG¹ and S ROSEHL²

¹Department of Surgery Huddinge sjukhus and ²Department of Pharmacology Karolinska Institute, Stockholm, Sweden

The aim of the present study was to compare the plasma levels of neurotensin like immunoreactivity (NTLI) after the oral administration of fat between healthy and obese volunteers and patients operated for obesity with jejuno-ileal bypass where a large proportion of the N-cell containing small intestine is shunted away from the contact with food.

Plasma NTLI was determined after the oral administration of 55 ml 70% Intralipid (11 g of fat mainly as triglycerides) to 3 groups of subjects:

(a) 8 healthy volunteers weighing on the average 62 kg

(b) 8 very obese but otherwise healthy volunteers weighing on the average 137 kg

(c) 20 patients who 7 to 10 years previously had been operated with jejuno-ileal bypass and there after decreased their average weight from 138 to 87 kg

After fat administration to the healthy volunteers the plasma concentration of NTLI increased significantly from 43 ± 5 pM to 85 ± 13 pM at 30 min. In the obese volunteers fat caused an increase of NTLI concentration from 27 ± 6 pM to a maximum of 50 ± 10 pM at 90 min. At 30 and 60 min after the fat administration the NTLI concentrations were significantly ($P < 0.01$) lower than for non-obese volunteers. In the patients with jejuno-ileal bypass the NTLI concentration increased from 34 ± 4 pM to a maximum of 125 ± 19 pM at 180 min which was significantly ($P < 0.01$) higher than in the obese volunteers.

These data indicate that plasma NTLI levels are

lower in very obese volunteers than in healthy ones. Fat induced increases in plasma NTLI were similar although delayed in obese subjects. Moreover 10 years after jejuno-ileal bypass the NTLI response was larger than in non-operated obese subjects. The reasons for these differences are not clear. The number of N-cells exposed to ingested fat may have been quite different in the obese and normal subjects. After jejuno-ileal bypass the number of N-cells may have increased in the functioning intestine which is well known to hypertrophy (Polak et al 1978, Besterman et al 1978). Alternatively the dose response curve may have been displaced to the left with increased sensitivity of the N-cell to fat. Another possibility is that some yet unknown substance released from the proximal intestine causes the increased response in plasma NTLI.

REFERENCES

- POLAK J M, HOBBS S F, BLOOM S R, BESTERMAN H S, GREENBERG G R, PEARES A O F, CLEARY J, GARLET J C, MONSON R & MILKINGTON R E 1978. Neurotensin and teroglucon in obesity. *Gastroenterology* 74 1079.
- BESTERMAN H S, SARSON D L, BLACKBURN A M, CLEARY J, MILKINGTON T R F, BLOOM S R 1978. The gut hormone profile in morbid obesity and following jejuno-ileal bypass. *Soc J Gastroenterol The 2nd International Symposium on Gastrointestinal Hormones* (ed J Myren, Schrupf L F, Hansen & M Vain) Vol 13, Sup 49 p 11.

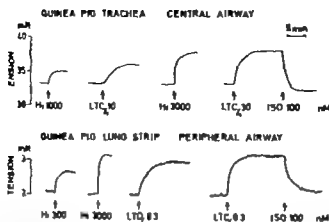


Fig. 3 Contraction responses to histamine (H), LTC_4 and LTD_4 in strips from guinea pig trachea and lung parenchyma. Reversal of LTC_4 -induced contraction by isoprenaline (ISO).

7 min) only after repeated washing or administration of the SRS-A antagonist FPL 55712 ($10 \mu\text{M}$).

The data presented in this communication show that the leukotrienes C and D are equally and probably potent in a number of organs. In particular, they are involved in anaphylactic reactions and they have a biological profile, which strikingly relates with that of impure preparations of S-A- Ac (Orange & Austen 1968). This strongly supports the concept that SRS-A consists of leukotenes LTC_4 and LTD_4 formed from arachidonic acid by the lipoxygenase pathway given in Fig. 1. LTC_4 is easily converted into LTD_4 by the plasma γ -glutamyl-transpeptidase and the relative proportions of these two leukotrienes in *in vitro* preparations therefore depend on incubation conditions (cf. Bach et al. 1979). Whether LTC_4 or LTD_4 is the principal mediator in anaphylactic reactions has not yet been established. However, there is not seem to be any significant difference in effects on the most sensitive respiratory muscles (preparations) i.e. the peripheral airways.

Supported by grants from the Swedish Medical Research Council (projects 84X-4342 and 03X-217), and from the National Institutes of Health.

REFERENCES

- BACH M. K., BRASHLER, J. R., BROOKS C. D. & NEERKEN A. J. 1979. Slow reacting substance. Comparison of some properties of human lung SRS-A and its distinct fractions from ionophore-induced rat mononuclear cell SRS. *J. Immunol.* 122, 160-165.
- BORGEAT P. & SAMUELSSON B. 1979. Arachidonic acid metabolism in polymorphonuclear leukocytes. Unstable intermediate in formation of dihydroxy acids. *Proc. Natl. Acad. Sci. (USA)* 76, 3213-3217.
- DRAZEN J. M., LEWIS R. A., WASSERMAN S. I., ORANGE, R. P. & AUSTEN K. F. 1979. Differential effects of partially purified preparation of slow-reacting substance of anaphylaxis on guinea pig tracheal spirals and parenchymal strips. *J. Clin. Invest.* 63, 1-5.
- MURPHY R. C., HAMMARSTRÖM S. & SAMUELSSON B. 1979. Leukotriene C. A slow reacting substance (SRS) from mouse mastocytoma cells. *Proc. Natl. Acad. Sci. (USA)* 76, 4275-4279.
- ORANGE, R. P. & AUSTEN K. F. 1969. Slow reacting substance of anaphylaxis. In: *Advances in Immunology* 10: 103-144.
- SAMUELSSON B., HAMMARSTRÖM S., MURPHY R. C. & BORGEAT P. 1980. Leukotrienes and slow reacting substance of anaphylaxis (SRS-A). *J. Allergy* 35, 373-381.
- STRANDBERG K. & HEDQVIST P. 1973. Airway effects of slow reacting substance, prostaglandin $\text{F}_{2\alpha}$ and histamine in the guinea pig. *Acta Physiol. Scand.* 94, 105-111.

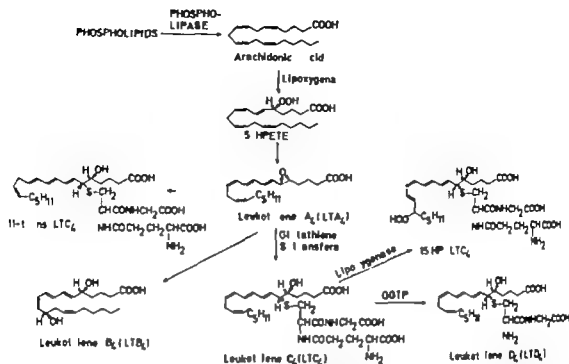


Fig. 1 Schematic representation of transformations of arachidonic acid into leukotrienes: 5-hydroperoxy-6,8,11-eicosatetraenoic acid (5-HPETE), 4(6)-oxido-7,9,11,14-eicosatetraenoic acid (LTA₄), 5(11)-dihydroxy-6,8,11-eicosatetraenoic acid (LTB₄), 5-hydroxy-6(8)-glutamylcysteinylglycyl-7,9,11,14-eicosatetraenoic acid (LTC₄), 5-hydroxy-6(8)-cysteinylglycyl-7,9,11,14-eicosatetraenoic acid (LTD₄), 5-hydroxy-15-hydroperoxy-6(8)-glutamylcysteinylglycyl-7,9,11,13-eicosatetraenoic acid (15-HP-LTC₄).

The pulmonary effects of LTC₄ and LTD₄ were further characterized in isolated strips from guinea pig, human and rabbit lung. As illustrated in Fig. 3 LTC₄ and LTD₄ were largely equipotent in con-

tracting guinea pig parenchymal or tracheal strip. The observation that lower concentrations of leukotrienes were required for significant contraction of parenchymal than tracheal strips, and that the histamine to leukotriene molar ratio for equivalent contraction amplitudes was more than 100:1 in tracheal strips and more than 1000:1 in parenchymal strips seems to indicate that leukotrienes are particularly active on peripheral airways, as previously suggested also for SRS-A (Drazen et al. 1977).

Rabbit bronchial strips did not respond to leukotrienes in concentrations up to 100 nM, whereas human bronchi again proved remarkably sensitive to LTC₄ and LTD₄. Thus, 0.1 nM of either leukotriene was sufficient for a significant response and 100% increase in tension was obtained with 6 nM LTC₄ as compared with 10 μM histamine. This would seem to make the leukotrienes outstanding bronchoconstrictors in man.

In all types of smooth muscle strips contraction induced by LTC₄ or LTD₄ invariably developed after a longer latency and at a considerably slower rate than those induced by histamine. Furthermore, they were consistently longlasting and unaffected by atropine (0.3 μM) or ramifen (1 μM), and

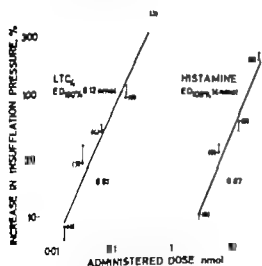


Fig. 2 Increase in insufflation pressure induced by intravenous injections of LTC₄ and histamine in guinea pigs. Shown are mean values \pm S.E. (n) = number of observations from 1 expts. Regression lines according to the method of least squares.

Free fatty acids in the brain in bicuculline-induced status epilepticus

CHAPMAN M. INGVAR and B. K. SIESJÖ*

Department of Experimental Brain Research, E-Blocket, University of Lund, Sweden

Although it is well known that prolonged or repeated epileptic seizures lead to neuronal cell damage in the brain, the mechanisms of damage have remained undefined. Experiments on bicuculline-induced status epilepticus in the baboon and the rat have shown that neuronal lesions develop in some selectively vulnerable areas (predominantly neocortex and hippocampus) in spite of artificial ventilation and a seemingly adequate oxygen supply (Mellgren et al 1973; Blennow et al 1978) and in spite of minimal perturbation of cerebral energy state (Chapman et al 1977). These results suggest that cell damage is at least partly unrelated to energy loss (see Blennow et al 1978). During recent years, it has become increasingly evident that cell damage may be elicited by oxidative breakdown of arachidonic acid via the cyclo-oxygenase (Larsson & Garbos 1975; Flower 1979) and lipoxygenase pathways (Borgeat & Samuelsson 1979). The question therefore arises whether arachidonic acid accumulates during status epilepticus. Previously elevated concentrations of free fatty acids (FFA) have been measured in seizures provoked by electro-shock and diazepam (see Bazán 1976; Marion & Wolfe 1976). However, since the animals used were not breathing spontaneously the possibility remains that respiratory distress contributed to the development of gross energy failure. In order to clarify whether or not FFA accumulate in the brain during seizures in the absence of such energy failure, we measured FFA concentrations in the cerebral cortex of rats in bicuculline-induced status epilepticus.

Methods. Male Wistar rats (250-400 g) were allowed free access to rat pellets and tap water. Anaesthesia was induced with halothane (2-3%), maintained during operative procedures on 1% halothane and 70% N₂O in oxygen, and then continued on 70% N₂O and 30% O₂ with artificial ventilation. Catheters were placed in femoral artery and

vein. EEG electrodes were inserted into the skull bone and provision were made for freezing the tissue *in situ*. Following adjustment of body temperature (37°C), arterial P_o (>100 mmHg) and P_{co} (35-40 mmHg), seizures were induced by i.v. injection of bicuculline (1.2 mg/kg). Cortical tissue was frozen for metabolite analyses after 1, 30, and 60 min of continuous seizure activity.

Frontoparietal cortical tissue from one hemisphere was extracted with HCl-methanol and subsequently with perchloric acid for enzymatic fluorometric analyses of adenosine nucleotides (ATP, ADP and AMP for references to analytical techniques, see Chapman et al 1977). A corresponding area from the other hemisphere was extracted with chloroform-methanol for the subsequent separation and analysis of FFA with thin layer and gas-liquid chromatography (for analytical techniques, see Retzlaff et al 1980).

Results and Discussion. The cortical FFA concentration in control animals was 0.16 ± 0.005 $\mu\text{mol/g}$ (mean \pm S.E., $n=6$). After 1, 30 and 60 min of seizure activity the concentrations rose to 0.365 ± 0.04 ($n=5$), 0.41 ± 0.041 ($n=6$) and 0.229 ± 0.15 $\mu\text{mol/g}$ respectively. As Fig. 1 shows the increase in arachidonic acid (20:4) and stearic acid (18:0) was even more pronounced while there were no significant changes in palmitic (16:0) or oleic (18:1) acids. The concentration of the polyenoic docosahexaenoic acid (22:6) rose 4- to 6-fold (data not shown).

At 1 min there was a moderate but clear perturbation of cerebral energy state with changes in ATP, ADP and AMP concentrations (cf. Chapman et al 1977). However, at 30 and 60 min cerebral energy state was minimally perturbed. For example, at 30 min the ATP, ADP and AMP concentrations were 2.62 ± 0.02 , 0.279 ± 0.011 and 0.041 ± 0.003 $\mu\text{mol/g}$ respectively while control concentrations were 2.77 ± 0.01 , 0.278 ± 0.009 and

* To whom correspondence should be directed.

Estimation of capillary permeability of inulin, sucrose and mannitol in rat brain cortex

JLE AMTORP

Institute of Medical Physiology A, University of Copenhagen, Denmark

AMTORP J. Estimation of capillary permeability of inulin, sucrose and mannitol in rat brain cortex. *Acta Physiol Scand* 1980 110: 337-342. Received 8 May 1979 [ISSN 0001-6772, Institute of Medical Physiology A, University of Copenhagen, Denmark].

The present study analyzed the brain uptake of the differently sized hydrophilic molecules ^{14}C -inulin, ^{14}C -sucrose and ^{14}C -mannitol. Constant tracer concentrations were maintained in blood plasma after renal ligation. Accumulation of the indicators was measured in brain cortex during the approach to steady state. At the conclusion of infusion periods of 5, 10, 15, 30 and 60 min, samples of cerebral cortex were analyzed for radioactivity. Fractions attributable to blood in the tissue were subtracted and time-dependent apparent distribution volumes of the indicators in the tissue were estimated. The brain level did not rise to more than 1% of that in the plasma for inulin and sucrose and to 6-7% for mannitol. The explanation for this could be a combination of restricted penetration from blood into brain and sink effect of cerebrospinal fluid (CSF). To determine the removal of the indicators into CSF, ventriculo-cisternal perfusion was performed during the period of tracer uptake in the tissue; the rate of passage of the indicators into perfusion fluid was found to be negligible in comparison to the rates of uptake in the tissue. As diffusion in the extracellular space could limit uptake rates from blood to brain, estimates of diffusion limited half-times were also made. Calculations showed that the major hindrance to indicator uptake in the tissue is located in the wall of the brain capillaries, thus the uptake data permit calculation of brain capillary permeability. The half-times for the distribution of the indicators in their equilibrated tissue distribution spaces were used to estimate brain capillary permeability. By comparison with the aqueous diffusion coefficients of the indicators it is concluded that the substances in the molecular weight range of 182 to 5900 daltons are subjected to restricted diffusion during the passage of the blood-brain barrier and that in addition non-discriminative transendothelial pathways are available.

Key words: ^{14}C Labelled inulin, sucrose and mannitol, blood-brain barrier permeability

Estimates of brain capillary permeability coefficients have been obtained by the aid of the indicator dilution method (Cronk 1965; *see also* *in vitro* studies, i.e. loss of material from blood as it flows through the brain in a single passage. This method is applicable to solutes with low rate of transcapillary transport due to avoidable inaccuracies in determining very low extractions (Lassen et al 1971). The only method which can be used involves tissue sampling at various times after a continuous vascular infusion (Patlak & Fenstermacher 1975).

In the present study the tissue uptake method (Johnson & Wilson 1966) based on measurements of uptake of solutes with time under conditions with constant concentration of indicator substance in blood, is used. Using radioactive indicators I have followed the permeation of cortical tissue by

inulin, sucrose and mannitol which all have small capillary permeability coefficients and which are essentially confined to the extracellular compartment.

The aim of the experiments was to obtain information on processes involved in the passage of the substances across the blood-brain barrier.

METHODS

Male Sprague-Dawley rats weighing 300-350 g were used. Details on surgical preparation and perfusion technique have been given previously (Amtorp 1979). In short, they were as follows: Anaesthesia was introduced by p-pentobarbital (50 mg/kg) and maintained by injections as required. Both kidneys were exposed by retroperitoneal approach and the renal pedicles ligated. Tracheostomy and intubation was made. Catheters were introduced into

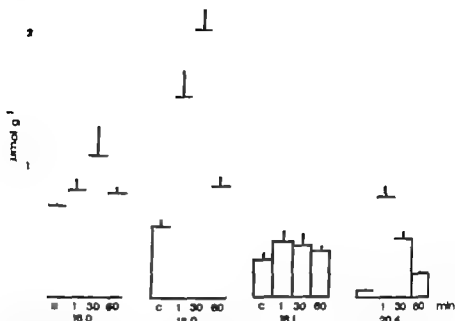


Fig. 1 Changes in the cerebral cortex concentrations of some free fatty acids during bicuculline-induced status epilepticus of 1, 30 and 60 min duration. The values are means \pm S.E. in μ mol/g of wet weight for groups of 6 animals. C = control. $P < 0.01$, $P < 0.001$.

0.044 ± 0.003 μ mol, respectively. A comparison with Fig. 1 shows that in spite of this near normal energy state the arachidonic acid concentration had increased more than 6-fold.

The results allow two main conclusions. First, a marked accumulation of arachidonic acid occurs and is maintained during seizures even though gross energy failure is absent. Second, since this accumulation occurs in a tissue whose oxygen supply is upheld, the conditions for an arachidonic acid cascade are at hand. Conceivably, oxidative metabolism of the arachidonic acid in the cycle-oxygenase and the lipoxygenase pathways contributes to the cell damage occurring.

This study was supported by grants from the Swedish Medical Research Council (project No. 14X 263) from US PHS (No. 5R01 NS07836) and from the Knut and Alice Wallenberg Foundation. The authors are grateful to Kerstin Behr and Lena Sjöberg for skilful technical assistance.

REFERENCES

- BAZÁN N. G. 1976. Free arachidonic acid and other lipids in the nervous system during early ischemia and after electroshock. In: *Function and metabolism of phospholipids in the central and peripheral nervous system* (ed. G. Porcellini, L. Amaducci and C. Gialli).

- Adv. Exp. Med. Biol. 72, 317-335. Plenum Press, New York/London.
- BLENNOV G., BRIERLEY J. B., MELDRUM B. S. & SIESJÖ B. K. 1978. Epileptic brain damage: The role of systemic factors that modify cerebral metabolism. *Brain* 101, 687-700.
- BORGEAT P. & SAMUELSSON B. 1979. Arachidonic acid metabolism in polymorphonuclear leukocytes. Effects of ionophore A₂₃₁₈₇. *Proc. Natl. Acad. Sci. USA* 76, 2148-2151.
- CHAPMAN A. G., MELDRUM B. S. & SIESJÖ B. K. 1977. Cerebral metabolic changes during prolonged epileptic seizures in rats. *J. Neurochem.* 28, 1025-1034.
- FLOWER R. J. 1979. Biosynthesis of prostaglandins. In: *Oxygen free radicals and tissue damage*. Ciba Foundation Symposium 83, pp. 123-138, 1979.
- MARION J. & WOLFE, L. S. 1978. Increase in the unesterified fatty acids, prostaglandin F₂, but not thromboxane B₂, in rat brain during drug-induced convulsions. *Prostaglandins* 16, 99-110.
- MARKELONIS G. & GARBUS J. 1975. Alteration of intracellular oxidative metabolism as stimulus evoking prostaglandin biosynthesis. *Prostaglandins* 10, 1007-1106.
- MELDRUM B. S., VIGOUROUX R. A. & BRIERLEY J. B. 1973. Systemic factors and epileptic brain damage. Prolonged seizures in paralyzed, artificially ventilated baboons. *Arch. Neurol.* 29, 82-87.
- REHNCRONA S., WESTERBERG E., ÅKESSON B. & SIESJÖ B. K. 1980. Brain cortical fatty acid and phospholipids during and following complete and severe incomplete ischemia. Submitted to *J. Neurochem.* in press.

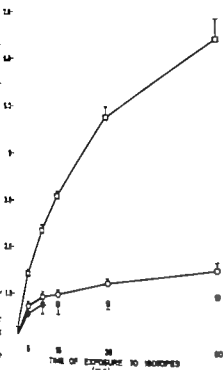


Fig. 1. The time course of uptake of inulin (●—●) sucrose (□—□) by rat cerebral cortex expressed as apparent spaces. Each point represents the mean value of 5 to 7 rats. The bars indicate ± 1 S.E.

expts in which ^{14}C -mannitol was infused for 60 min, 50 plasma samples were available. Taking the mean concentration in each expt. as 1.0 the regression of the concentration upon time was 1.022 ± 0.0002 (S.E. = 0.018) for inulin, 1.004 ± 0.0013 (S.E. = 0.022) for sucrose and 1.027 ± 0.0010 (S.E. = 0.013) for mannitol.

The uptake by brain cortex of ^{14}C -inulin, ^{14}C -sucrose and ^{14}C -mannitol expressed as apparent spaces versus time of exposure to the indicators is given in Fig. 1.

In Table 1 are presented the rate constants for the penetration from blood to brain cortex of ^{14}C -inulin, ^{14}C -sucrose and ^{14}C -mannitol obtained as described. The relatively low correlation coefficients of the regression lines calculated for ^{14}C -inulin and ^{14}C -sucrose are due to the low slopes of the regression lines. Also included are the values of equilibrium extracellular distribution volumes in cortical tissue (V_{ex}). The capillary permeability-surface area products (PS) as the best working measure of bar-

rier permeability were calculated from the rate constants and equilibrium distribution volumes.

DISCUSSION

In this study an attempt was made to determine brain capillary permeability to molecules which have small permeability coefficients. The method used and its application to various tissues has been discussed in detail (Johnson & Wilson 1966; Johnson 1970; Wittmers et al. 1976).

When considering the kinetics of uptake by cortical tissue several problems may complicate the analysis of the data. The choice of inulin, sucrose and mannitol as test substances which are inert of nature avoids problems concerned with influence of chemical processes upon the kinetics of uptake by the tissue. The contents of the indicators in cortical tissue was obtained by subtraction of residual amounts of inulin, sucrose and mannitol in the vascular compartment, which allowed calculation of the tissue volumes of distribution at the time the sample was taken. Correction was obtained in the individual experiments by use of ^{125}I -indium bound to plasma transferrin as a plasma tracer. By this procedure the vascular plasma compartment was found to be 0.44 μl /100 mg with a variation of 6%.

A substance passing out of the blood capillaries first enters the extracellular space and from there it will pass into the cells if the cell membranes are permeable to the substance. The particular substances being studied here are essentially confined to the extracellular compartment and the uptake by brain cortex was considered to occur exclusively in the extracellular fluid in the time periods studied. The equilibrium extracellular volumes of distribution in rat brain cortex have been measured recently (Amlorp 1979). The distribution spaces of ^{14}C -inulin, ^{14}C -sucrose and ^3H -mannitol were determined following tracer equilibration times of 5 h with combined intravenous infusion and ventriculo-cisternal perfusion, the aim being to preclude sink action of csf during equilibration (Oldendorf & Davson 1967). The variability (S.D. 100/mean) of the equilibrium volumes of distribution was of the order of 7% for ^{14}C -inulin and of the order of 13% for ^{14}C -sucrose and ^3H -mannitol. It is evident that there was some scatter in these data, which affects the accuracy of the estimates of capillary permeability.

Opportunities exist for diffusional exchange of solutes between brain extracellular fluid and csf

a femoral artery and vein. The arterial blood pressure was continuously recorded. Body temperature was maintained at 37.8–38.2°C. The animals were immobilized with gal lamonium iodide (70 mg/kg). Artificial ventilation with ambient air was maintained with a small animal respirator and pH P_{aO_2} and P_{aCO_2} were measured (BMS 3 MK, Blood Micro System Radiometer Copenhagen).

With the aid of a stereotactic device (Kopf Instruments) two inflow cannulae were placed, one in each lateral ventricle. The ventricular system of the brain was perfused with artificial csf. The rate of infusion delivered from a slow infusion pump (Braun-Melungen) was 15 μ l/min with one half diverted to each lateral ventricle. The atlanto-occipital membrane was exposed and a cisternal cannula was inserted into the cisterna magna. Outflow fluid was collected via polyethylene tubing into glass vials placed close to the experimental animals in order to minimize the dead space, i.e. the perfusion volume between cisterna magna and the test tubes (estimated to less than 700 μ l). Collection of fluid was carried out under a negative pressure of 10 cm H $_2$ O, the aim being to keep the pressure in csf in the subarachnoid space below that in the venous sinuses.

Inulin, sucrose and mannitol were the test molecules utilized in the experiments. 14 C-labelled inulin, sucrose and mannitol were obtained from New England Nuclear Corp., Boston, Mass., USA. The purity of isotopical inulin, sucrose and mannitol was verified by gel filtration using a G-25 Sephadex column (Pharmacia). The isotopic test molecules were prepared in isotopic NaCl.

The volume of residual blood in the brain was estimated using 125 I-indium bound to plasma transferrin as a plasma tracer (Hosokawa et al. 1969). Freshly generated 125 I-indium obtained from a sterile ion exchange generator was mixed with arterial blood and injected i.v. 5 min before the end of the experiments.

To determine the time course of permeation of the indicator molecules from plasma into brain, a solution of the test substance was infused into a femoral vein. In preliminary experiments on rats with ligated renal vessels, plasma clearance of 14 C-labelled inulin, sucrose or mannitol was determined from multiple small plasma samples obtained at variable time intervals between 1 min and 1 h after i.v. injection. Using the plasma clearance curves, an empirical infusion schedule was designed to give approximately constant plasma concentration.

Sampling of tissue and fluids. The effluent csf was collected continuously during periods of 5 min and effluent volumes were determined by weighing. At a set time, samples of arterial blood plasma were assayed for radioactivity and the animals were killed by decapitation and exsanguinated to minimize the vascular blood content of the brain. The brain was rapidly removed and divided into separate hemispheres. The layer of cerebral cortex was separated by peeling off subcortical tissues and careful removal of the meninges.

Analyses. A Packard Tri-Carb liquid scintillation spectrometer system, model 2425, was used to count carbon-14 labelled compounds. 125 I-indium ($t_{1/2}$ = 99.5 min) was allowed to decay before beta counting commenced. 25 μ l plasma and 50 μ l perfusion fluid were placed in scintillation vials. Pieces of brain were weighed in scintillation

vials. Beta counting was performed after tissue solubilization. 10 ml scintillator solution (Instagel, Packard) was added to all the vials, which were kept in darkness at 4°C for 1 h to eliminate chemiluminescence. Quench correction was calculated using external standard channel ratio method. Gamma emission from 125 I-indium was counted in a well crystal counter (Packard Autogamma, model 584) and correction for decay during the counting period was made.

Calculations. After appropriate background and efficiency corrections, brain tissue activity of the test molecule (cpm/g wet weight) was obtained and the fraction attributable to blood remaining in the brain was subtracted. The apparent extravascular distribution volume in the tissue (V_e) was then calculated as:

$$V_e = \frac{\text{activity per 100 mg tissue}}{\text{activity per } \mu\text{l plasma water}} (\mu\text{l} (100 \text{ mg})^{-1})$$

The values of equilibrium extracellular distribution volumes (V_{eq}) of the indicators in brain cortex were obtained from a recent study (Amtrup 1979). The rate constants for penetration of the solutes into brain were obtained by calculating the regression lines for the ratio $\ln(1/V_e/V_{eq})$ vs. time. The permeability surface area product (PS) with dimensions of length 3 time $^{-1}$ mm $^{-1}$ equals the product of the rate constant (k) and the equilibrium volume of distribution (V_{eq}) (Johnson & Wahren 1966, Wahren et al. 1976).

$$PS = k \cdot V_{eq} (\text{ml} (100 \text{ g})^{-1} \text{ min}^{-1}) \quad (1)$$

The assumptions underlying this equation are: (1) Homogeneity of exchange vessels with respect to permeability. (2) The resistance to diffusional distribution of indicator material within the tissue distribution space is much lower than that offered by the endothelium of the exchange vessels, i.e. the tissue distribution space can be considered well-mixed. (3) Indicator material cannot leave the tissue space by any other routes than the vascular endothelium through which it has entered the tissue. The last assumption implies that passage of indicator material from the tissue to csf must be negligible.

RESULTS

Tidal volume and rate of respiration was adjusted using a constant volume respirator in order to keep arterial carbon dioxide pressure (P_{aCO_2}) between 30 and 40 torr. Arterial blood pressure averaged 98 mmHg (range 75–135 mmHg).

The concentration of 14 C inulin, 14 C sucrose and 14 C mannitol attained in blood plasma of nephrectomized rats was analysed in preliminary experiments. In 5 expts in which 14 C inulin was infused for 60 min, 53 samples of plasma were available in 5 expts in which 14 C sucrose was infused for 60 min, 54 plasma samples were available and in 5

Table 3 Physical parameters of *in vivo* molecules

The diffusion coefficient in water at 38°C was calculated from D_w by the relation:

$$D_w = D(11.253/r)^2$$

where r is the radius of water at indicated temperature and D is the diffusion coefficient in water at 38°C. R is the gas constant, T absolute temperature in °K and N Avogadro's number.

Compound	D_w cm ² ·sec ⁻¹ · 10 ⁶	Hydrodynamic molecular radius cm · 10 ⁸
Inulin	2.5	13.1
Sucrose	7.0	4.6
Mannitol	8.8	3.7

the conclusion that the rate limiting step of the passage of the substances from blood into cortical tissue is transfer across the capillary membrane.

The calculation of brain capillary permeability from the time course of uptake of indicators into brain cortex is based upon the basic assumption that the rate of passage of the indicators is at any time proportional to their concentration differences across the blood-brain barrier, i.e. that the passage occurs by diffusion and/or bulk transport symmetrically with respect to the barrier. By use of differently sized indicator molecules present data may contribute to our understanding of mechanisms of transcapillary movement such as non-restricted diffusion or restricted diffusion. Non-restricted diffusion through water-filled channels implies that indicator molecules with different molecular size diffuse across the capillary membrane at rates proportional to their respective aqueous diffusion coefficients. The concept of restricted diffusion implies a deviation of the ratio between the permeability coefficients from the ratio between the aqueous diffusion coefficients. Some physical parameters of the indicator molecules are presented in Table 3. The ratio $D(\text{mannitol})/D(\text{sucrose}) = 1.22$ and the ratio $D(\text{mannitol})/D(\text{inulin}) = 3.52$. The corresponding ratio between the calculated PS-values were 4.90 and 8.01 respectively. These PS ratios are not commensurate with non-restricted diffusion in water-filled channels through or between capillary endothelial cells. Permeability decreased disproportionately with increasing molecular size the quoted ratio of permeability coefficient being larger than

the ratios of corresponding aqueous diffusion coefficients suggesting that entry of the solutes into brain cortex is governed by a sieving process. However the ratio of permeability coefficients between ^3C sucrose and ^3C -inulin was lower than the ratio between the aqueous diffusion coefficients which is incompatible with restricted diffusion through aqueous pores as the only route of passage of the substances across the blood-brain barrier. This is so whether the barrier has pores of uniform or distributed size. A more complex model such as one with pores plus bulk transport in vesicles is required to explain the data (cf. Garlick & Renkin 1970; Carter et al. 1974).

The most likely interpretation of the experimental data is that there are two pathways for the passage of the substances: a set of pores which allows passage by restricted diffusion and a non-discriminative process. The former would account for the major part of the passage of mannitol while the latter would be responsible for a considerable part of the passage of sucrose and, in particular, of inulin. The non-discriminative process probably consists of a set of pinocytotic vesicles which open to the luminal surface of the capillary membrane during formation and include indiscriminately in the vesicular volume anything that is originally in solution in the plasma. Thereafter the vesicles exchange their contents by transport across the endothelial cells. The motion of free vesicles within the cytoplasm of the endothelial cells is assumed to be Brownian (Karnovsky & Skea 1970). The contribution of vesicular transport to solute permeability equals the rate of turn-over of the vesicles (Renkin 1964; Palade & Burris 1968; Tonin 1969; Shea et al. 1969).

I wish to express my appreciation to Mrs Jytte Antorp for her expert technical assistance.

Supported in part by the Warburg Larsen Foundation, Denmark.

REFERENCES

- ANTORP O. 1979 Distribution of inulin, sucrose and mannitol in rat brain cortex slices following *in vivo* or *in vitro* equilibration. *J. Physiol.* 294: 81-90.
- BRADBURY M. 1979 Introduction to the blood-brain-cerebrospinal fluid system. 1a. The concept of blood-brain barrier pp. 3-36. W.D. Chichester New York, Brisbane, Toronto.
- CARTER R. D., JOYNER W. L. & RENKIN E. M. 1974 Effects of histamine and some other substances

Table 1 *Capillary permeability surface area products (PS)*

In the first column are given the compounds to which the values in the same row of the subsequent columns are related. The second column gives rate constants for the uptake of the compounds. The third column gives correlation coefficients for linear regression lines from which rate constants for uptake were determined. The fourth column gives the equilibrium volumes of distribution. The fifth column gives the calculated permeability surface area products.

Compound	k (min ⁻¹)	r	V _{eq} (ml/100 g)	PS (ml/100 g min)
¹⁴ C Inulin	0.0011	0.83	13.3	0.015
¹⁴ C Sucrose	0.001	0.84	16.6	0.020
¹⁴ C Mannitol	0.006	0.97	19.5	0.121

which may contribute to underestimate indicator uptake by brain cortex and should be taken into account (Welch 1969). The total volume of csf is relatively small, about 10% of brain weight (Bradbury 1979). It is in contact with the central nervous tissue over considerable areas of the ependymal and pia glial membranes. In the rat pia and arachnoid are in opposition in some places, but in others separate to enclose cisterns of csf. Over the cerebral cortex these cisterns are absent and subarachnoid csf should not be regarded as bathing the whole of the external surface of the cortex (Bradbury 1979). The flow of csf in cerebral ventricles and subarachnoid space is governed by small hydrostatic pressure gradients generated by its production in the choroid plexus. The drainage of csf is largely via the arachnoid villi (Davson et al. 1970). During ventriculo-cisternal perfusion with pressure about -100 cm H₂O the arachnoid villi collapse (Pappenheimer et al. 1962). In the present experiments csf in juxtaposition to the cerebral cortex should therefore be rather stagnant and only able to leave by way of the outflow cannula in the cisterna magna. Analysis of the indicator contents in the outflow fluid allowed estimation of the overall losses by way of csf. Accumulation of indicator material in outflow perfusion fluid during ventriculo-cisternal perfusion was compared with accumulation in the tissue during periods of tracer uptake. It was found that the removal by the perfusion fluid amounted to only 5-7% of indicator substance accumulated in brain cortex. Since a major part of indicator material entering the perfusion fluid by passage into the ventricular cavities must arise from blood passing vessels in the vicinity of the ventricles and possibly the choroid plexus, the sink effect of perfusion fluid and subarachnoid csf with respect to the cerebral cortex must be considered negligible.

In order to quantitate the indicator uptake data in terms of capillary permeability, some assumptions were made about the system (Johnson 1970). Rad mixing of the indicator material within the capillaries seems fulfilled in this study, since capillary diameter of 4.8×10^{-4} cm (Wiederbold et al. 1974) and diffusion coefficients are relatively large ($> 2 \times 10^{-6}$ cm² sec⁻¹). Diffusional mixing in the extravascular distribution volume was evaluated by an equation employed by Schafer & Johnson (1971) which yields an expression for diffusion and half times

$$t_{1/2} = 0.693 (RV^2/AD^2)$$

The apparent diffusion coefficients (D^*) for the indicator molecules in brain tissue were taken from Fenstermacher et al. (1970). Available data for capillary surface area ($A = 740$ cm² g⁻¹) and maximum diffusion distance ($R = 16 \cdot 10^{-3}$ m (Sjoststrand 1935)) were used. Table 2 summarizes diffusion limited half times calculated from equation (2) and the half times for tracer uptake from capillary blood calculated from present experimental data. A comparison of the half-times support

Table 2 *Estimated half times for diffusion equilibration in brain tissue and the half-times for cortical uptakes calculated from present experimental data*

Compound	Diffusion $t_{1/2}$ (min)	Observed $t_{1/2}$ (min)
¹⁴ C Inulin	118	626
¹⁴ C Sucrose	11	591
¹⁴ C Mannitol	13	11

Table 3. Physical parameters of test molecules

The aqueous diffusion coefficient at 37°C was calculated from D_0 by the relation

$$D_{37} = D_0 (311/293)^{1/2} (\eta_{20}/\eta_{37})$$

where η is the viscosity of water at indicated temperature, D_0 Einstein hydrodynamic radius = $RT/6\pi\eta DV$ the gas constant, T absolute temperature, V molar volume, D free diffusion coefficient of solute and N Avogadro number

Compound	D_0 cm ² sec ⁻¹ $\times 10^6$	Hydrodynamic molecular radius cm $\times 10^8$
mannitol	2.5	13.1
sucrose	7.2	4.6
inulin	111	3.7

The conclusion that the rate limiting step of the passage of the substances from blood into cortical tissue is transfer across the capillary membrane

The calculation of brain capillary permeability from the time course of uptake of indicators into brain cortex is based upon the basic assumption that the rate of passage of the indicators is at any time proportional to their concentration differences across the blood-brain barrier (i.e. that the passage occurs by diffusion and/or bulk transport symmetrically with respect to the barrier. By use of differently sized indicator molecules present data may contribute to our understanding of mechanisms of transcapillary movement such as non-restricted diffusion or restricted diffusion. Non-restricted diffusion through water-filled channels implies that indicator molecules with different molecular size diffuse across the capillary membrane at rates proportional to their respective aqueous diffusion coefficients. The concept of restricted diffusion implies a deviation of the ratio between the permeability coefficients from the ratio between the aqueous diffusion coefficients. Some physical parameters of the indicator molecules are presented in Table 3. The ratio D (mannitol)/ D (sucrose) = 1.22 and the ratio D (mannitol)/ D (inulin) = 3.52. The corresponding ratio between the calculated PS-values were 6.70 and 10.0 respectively. These PS ratios are not commensurate with non-restricted diffusion in water-filled channels through or between capillary endothelial cells. Permeability decreased disproportionately with increasing molecular size the quoted ratios of permeability coefficients being larger than

the ratios of corresponding aqueous diffusion coefficients suggesting that entry of the solutes into brain cortex is governed by a sieving process. However the ratio of permeability coefficients between ¹⁴C-sucrose and ¹⁴C inulin was lower than the ratio between the aqueous diffusion coefficients which is incompatible with restricted diffusion through aqueous pores as the only route of passage of the substances across the blood-brain barrier. This is so whether the barrier has pores of uniform or distributed size. A more complex model such as one with pores plus bulk transport in vesicles is required to explain the data (cf. Garlick & Renkin 1970; Carter et al. 1974).

The most likely interpretation of the experimental data is that there are two pathways for the passage of the substances: a set of pores which allows passage by restricted diffusion and a non-discriminative process. The former would account for the major part of the passage of mannitol while the latter would be responsible for a considerable part of the passage of sucrose and in particular of inulin. The non-discriminative process probably consists of a set of pinocytotic vesicles which open to the luminal surface of the capillary membrane during formation and include indiscriminately in the vesicular volume anything that is originally in solution in the plasma. Thereafter the vesicles exchange their contents by transit across the endothelial cells. The motion of free vesicles within the cytoplasm of the endothelial cells is assumed to be Brownian (Karnovsky & Shea 1970). The contribution of vesicular transport to solute permeability equals the rate of turn-over of the vesicles (Renkin 1964; Palade & Burns 1968; Tomlin 1969; Shea et al. 1969).

I wish to express my appreciation to Mrs Jytte Amtrup for her expert technical assistance.

Supported in part by the Warvar Larsen Foundation, Denmark.

REFERENCES

- AMTRUP O 1979 Distribution of inulin, sucrose and mannitol in rat brain cortex slices following *in vivo* or *in vitro* equilibration. *J Physiol* 294: 81-90.
- BRADBURY M 1979 Introduction to the blood-brain-cerebrospinal fluid system. 1st: The concept of blood-brain barrier pp 3-36 Wiley Chichester New York, Brisbane, Toronto.
- CARTER, R. D., JOYNER, W. L. & RENKIN, E. M. 1974 Effects of brinsamine and some other substances

- on molecular selectivity of the capillary wall to plasma proteins and dextran. *Microvasc Res* 7: 31-38.
- CRONE, C. 1965. The permeability of brain capillaries to non-electrolytes. *Acta Physiol Scand* 64: 407-417.
- DAVSON, H., HOLLINGWORTH, G. & SEGAL, M. B. 1970. The mechanism of drainage of the cerebrospinal fluid. *Brain* 93: 665-678.
- FENSTERMACHER, J. B., RALL, D. P., PATLAK, C. B. & LEVIN, V. A. 1970. Ventriculo-cerebral perfusion as a technique for analysis of brain capillary permeability and extracellular transport. In: *Capillary permeability*. Alfred Benzon Symp. II (ed. C. Crone and N. A. Lassen) pp. 483-490. Munksgaard, Copenhagen.
- GARLICK, P. G. & RENKIN, E. M. 1970. Transport of large molecules from plasma to interstitial fluid and lymph in dogs. *Amer J Physiol* 19: 1595-1605.
- HOSSAIN, P., MCINTYRE, A., POULOSE, K., STERN, H. S. & WAGNER, N. H. Jr. 1969. Binding of trace amounts of iodic iodine-113m to plasma transferrin. *Clin Chim Acta* 4: 69-75.
- JOHNSON, J. A. 1970. A model for capillary exchange with applications. In: *Capillary permeability*. Alfred Benzon Symp. II (ed. C. Crone and N. A. Lassen) pp. 706-714. Munksgaard, Copenhagen.
- JOHNSON, J. A. & WILSON, T. A. 1966. A model for capillary exchange. *Amer J Physiol* 10: 1799-1803.
- KARNOVSKY, M. J. & SHEA, M. S. 1970. Transcapillary transport by pinocytosis. *Microvasc Res* 3: 353-360.
- LASSEN, N. A., TRAPJENSEN, J., ALEXANDER, S. G., OLESEN, J. & PAULSON, O. B. 1971. Blood-brain barrier studies in man using the double-indicator method. *Amer J Physiol* 220: 1627-1633.
- OLDENDORF, W. H. & DAVSON, H. 1967. Brain extracellular space and the sink action of cerebrospinal fluid. *Arch Neurol* 17: 196-205.
- PALADE, G. E. & BURNS, R. R. 1968. Structural modification of plasmalemmal vesicles. *J Cell Biol* 37: 671-649.
- PAPPENHEIMER, J. R., HEISEY, S. R., KORDA, E. F. & DOWNER, J. de C. 1966. Perfusion of the cerebral ventricular system in unanesthetized rats. *Am J Physiol* 210: 767-774.
- PATLAK, C. B. & FENSTERMACHER, J. D. M. 1971. Comparison of methods for determining blood barrier permeability. In: *Fluid environment of the brain* (ed. H. F. Cserr, J. D. Fenstermacher & F. F. pp. 43-59. Academic Press, New York, San Francisco, London).
- RENKIN, E. M. 1964. Transport of large molecules across capillary walls. *Physiol Rev* 44: 12-21.
- SCHAFER, D. M. & JOHNSON, J. A. 1964. Permeability of mammalian heart capillaries to sucrose and inulin. *Amer J Physiol* 206: 985-991.
- SHEA, M. S., KARNOVSKY, M. J. & BOSSERT, E. B. 1969. Vesicle transport across endothelium. Simulation of a diffusion model. *J Theor Biol* 4: 30-42.
- SJOSTRAND, T. 1935. On the principles for distribution of blood in the peripheral vascular system. *Scand J Clin Lab Invest* 81: 1-140.
- TOMLIN, W. G. 1969. Vesicular transport across endothelial cells. *Biochem Biophys Acta* 183: 449-464.
- WELCH, R. 1969. A model for the distribution of materials in the fluids of the central nervous system. *Brain Res* 16: 453-468.
- WEDERHOLD, A. B., BIELZER, W. Jr, SCHULZ, V., VETEAU, M. J. & HUNZIKER, O. 1976. Three-dimensional reconstruction of brain capillaries from frozen serial sections. *Microvasc Res* 11: 175-180.
- WITTMERS, L. E., BARLETT, M. & JOHNSON, J. A. 1976. Estimation of capillary permeability coefficient of inulin in various tissues in the rabbit. *Microvasc Res* 11: 67-78.

Adjustments of hepatic and small intestine blood flow on selective vasoconstrictor fibre stimulation

FASTH L, HULTÉN S and NORDGREN S

Department of Surgery II, Sahlgrenska sjukhuset, University of Göteborg, Sweden

FASTH S, HULTÉN L & NORDGREN S. Adjustments of hepatic and small intestinal blood flow on selective vasoconstrictor fibre stimulation. *Acta Physiol Scand* 1980; 110: 343-350. Received 16 Jan. 1980. ISSN 0001-6772. Department of Surgery II, Sahlgrenska sjukhuset, University of Göteborg, Sweden.

The mutual changes in hepatic and small intestinal blood flow on selective nervous stimulation of the perarterial vasoconstriction fibres, were studied in anesthetized cats. Occlusion of the hepatic artery did not change portal blood flow whereas occlusion of superior mesenteric blood flow caused a significant increase in hepatic arterial flow. Stimulation of the hepatic sympathetic nervous supply caused phasic blood flow response with marked transient peak flow reduction of hepatic arterial blood flow. The amplitude of the peak response varied with the frequency of the stimulation. Despite continuous stimulation the first phase went over into second phase of less pronounced vasoconstriction. This steady state blood flow was maintained at about 20% below the control level, irrespective of stimulation frequency. Corresponding to time with the peak vasoconstriction there was transient increase of portal pressure. Sympathetic nerve stimulation increased portal pressure even on occlusion of the hepatic artery. Stimulation of the mesenteric sympathetic nerves evoked the characteristic transient peak vasoconstrictor response consisting of two phases, brief haemo peak resistance response followed by a second phase of less pronounced but generally well maintained constriction. Simultaneously a slight reduction of portal pressure and hepatic arterial vascular resistance was regularly seen. In contrast to the observations in the hepatic arterial circuit the magnitude of the steady state blood flow in the small intestine was dependent on the rate of the stimulation, however. On simultaneous stimulation of the hepatic and mesenteric sympathetic nerves the hemodynamic responses were largely the same as when these nerves were stimulated separately. The portal pressure affecting mean capillary pressure in the intestine differed, however. Small and variable pressure changes were followed by rapid return towards control and during steady state it did not differ from the prestimulatory level.

Many studies on the inherent myogenic and sympathetic nervous control of hepatic and intestinal blood flow have been reported (For review, see Brauer 1963; Folkow & Neil 1971; Greenway & Stark 1971). Common for these studies are that each vascular bed has been studied separately and don't permit conclusions regarding the intimate interaction between intestinal and hepatic blood flow such as it occurs during simultaneous graded stimulation of the sympathetic nerves to both vascular beds.

The purpose of the present investigation was to study qualitatively and quantitatively the mutual changes of hepatic and small intestinal blood fol-

lowing selective graded electrical stimulation of the perarterial vasoconstrictor fibres, supplying these vascular circuits. It was also considered of interest to study changes in the blood flow and portal vein pressure during vasoconstrictor fibre activation when either or both circuits were temporarily occluded.

METHODS

Operative procedures. Experiments were performed on 20 cats weighing 4-4.4 kg. The animals, fasted for 24 h, were anesthetized with chloralose (50-70 mg/kg) after induction with ether. A tracheal cannula was inserted to maintain free airway. The abdomen was opened in

- on molecular selectivity of the capillary wall to plasma proteins and dextran. *Microvasc Res* 7: 31-38.
- CRONE C. 1965. The permeability of brain capillaries to non-electrolytes. *Acta Physiol Scand* 64: 407-417.
- DAVSON H, HOLLINGWORTH G & SEGAL, M B. 1970. The mechanism of drainage of the cerebrospinal fluid. *Brain* 93: 665-678.
- FENSTERMACHER J D, RALL D P, PATLAK C S & LEVIN V A. 1970. Ventriculo-cisternal perfusion as a technique for analysis of brain capillary permeability and extracellular transport. In: *Capillary permeability* Alfred Benzon Symp II (ed C Crone and N A Lassen) pp 483-490. Munksgaard Copenhagen.
- GARLICK P G & RENAIN E M. 1970. Transport of large molecules from plasma to interstitial fluid and lymph in dogs. *Amer J Physiol* 19: 1594-1605.
- HOSSAIN P, MCINTYRE A, POULOSE, K, STERN H S & WAGNER N H Jr. 1969. Binding of trace amounts of ionic Iodine-125 in plasma transferrin. *Clin Chim Acta* 24: 69-75.
- JOHNSON J A. 1970. A model for capillary exchange with applications. In: *Capillary permeability* Alfred Benzon Symp II (ed C Crone and N A Lassen) pp 206-214. Munksgaard Copenhagen.
- JOHNSON J A & WILSON T A. 1966. A model for capillary exchange. *Amer J Physiol* 10: 1299-1303.
- KARNOVSKY M J & SHEA M S. 1970. Transcapillary transport by pinocytosis. *Microvasc Res* 2: 353-360.
- LASSÉN N A, TRAP-JENSEN J, ALEXANDER S G, OLESEN J & PAULSON O B. 1971. Blood-brain barrier studies in man using the double-indicator method. *Amer J Physiol* 220: 1627-1633.
- OLDENDORF W H & DAVSON H. 1967. Brain extracellular space and the sink action of cerebrospinal fluid. *Arch Neurol* 17: 196-205.
- PALADE G E. & BURNS R R. 1966. Structural localization of plasmalemmal vesicles. *J Cell Biol* 37: 617-649.
- PAPPENHEIMER J E, HEISEY S R, JORDAN F & DOWNER, J de C. 1964. Perfusion of the cerebral ventricular system in anesthetized man. *Am J Physiol* 30: 763-774.
- PATLAK C S & FENSTERMACHER J D. 1971. Comparison of method for determining blood-brain barrier permeability. In: *Fluid environment of the brain* (ed H F Cserr, J D Fenstermacher, V Fed) pp 48-59. Academic Press, New York, San Francisco, London.
- RENKIN E M. 1964. Transport of large solutes across capillary walls. *Physiological* 7: 13-25.
- SCHAFER D M & JOHNSON J A. 1964. Permeability of mammalian heart capillaries to sucrose and inulin. *Amer J Physiol* 206: 985-991.
- SHEA M, KARNOVSKY M J & BOSSERT W J. 1969. Vesicle transport across endothelium. Simulation of a diffusion model. *J Theor Biol* 4: 30-44.
- SJÖSTRAND T. 1935. On the principles for distribution of blood in the peripheral vascular system. *Scand Arch Physiol* 81: 1-150.
- TOMLIN W G. 1969. Vesicular transport across endothelial cells. *Biochem Biophys Acta* 183: 549-564.
- WELCH K. 1969. A model for the distribution of solutes in the fluids of the central nervous system. *Br Res* 16: 453-468.
- WIEDERHOLD K H, BIELZER W Jr, SCHUL V, VETEAU M J & HUNZIKER, O. 1976. The dimensional reconstruction of brain capillaries from frozen serial sections. *Microvasc Res* 11: 175-180.
- WITTMERS L F, BARLETT M & JOHNSON J J. 1976. Estimation of capillary permeability coefficient of inulin in various tissues in the rabbit. *Microvasc Res* 11: 67-78.

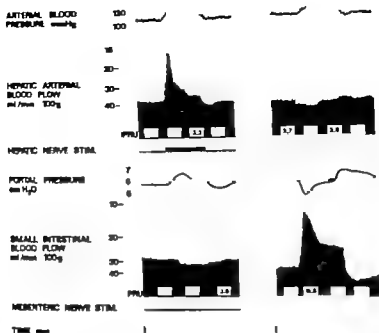


Fig. 2. Cat 3.1 kg. The effects of hepatic (left panel) and mesenteric (right panel) nerve stimulation (8 Hz, 5 ms, 1 V) on systemic arterial blood pressure, hepatic arterial blood flow, portal pressure and small intestinal blood flow. Note the more short-lasting peak response and the more pronounced escape from the vasoconstrictor activation in the hepatic vascular bed. Note also that the portal pressure approaches the control level during sustained stimulation.

blood flow to about 100 ml/min $\times 100$ g tissue. No significant change in portal pressure was observed. When administered in the superior mesenteric artery isoprenaline increased small intestinal blood flow to 200–250 ml/min $\times 100$ g tissue and the portal pressure to about 100% of the control value, which ranged between 2.9–1 cm H₂O (mean 5.8 ± 0.2). The blood flow levels correspond to those previously reported on cats where enous circuits were used (Folkow, Lundgren & Wallentin 1963; Greenway, Larsson & Mellander 1967), indicating a well-maintained myogenic tone in the present series of experiments despite the use of arterial circuits. In the present preparation the portal blood flow equivalent to the superior mesenteric arterial blood flow is calculated to 37 ± 2 ml/min $\times 100$ g liver tissue. Except for the pancreas the blood flow in the stomach and the colon when studied by enous or arterial circuits is somewhat lower than in the small intestine and amounts to about 70 ml/min $\times 100$ g tissue (Marlinton 1965; Hultén 1969; Fasth & Hultén 1973; Fasth & Marlinton 1973). The resting portal blood flow in the anesthetized cat with the splanchnic vascular bed intact can be calcu-

lated roughly to about 80 ml/min $\times 100$ g liver tissue. The corresponding reduction of the portal flow in the present study seems to affect the magnitude of the portal pressure but little as this has been reported to range between 5–15 cm H₂O in anesthetized cat with almost intact splanchnic circulation (Greenway et al 1967).

11 The effects of graded sympathetic nerve stimulation

A. Hepatic nerves. The neurogenic constriction of the hepatic resistance vessels exhibited two phases. The threshold value for evoking a response was 0.5 Hz. As is shown in Fig. 2 (left panel), there was first a brief but intense reduction of hepatic arterial blood flow (peak response). The magnitude of the initial peak flow reduction showed a linear correlation to the logarithm of the stimulation frequency ($r = 0.70$), 16 Hz causing almost circulatory arrest (Fig. 3 (left panel)). When calculated as changes in PRU₁₀₀, the peak response at 8 Hz and 16 Hz corresponded to an increase of resistance to flow amounting to 600–1000%. Despite continuous stimulation this initial flow reduction subsided after 20–30 and

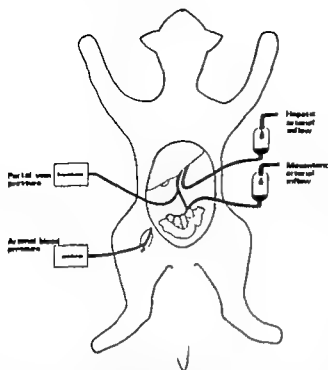


Fig. 1 Schematic illustration of the technique used for synchronous studies of small intestinal and hepatic arterial blood flow. Note that small intestinal blood flow reflects portal flow.

the midline and the greater omentum the spleen the stomach the duodenum the pancreas and colon were extirpated. To avoid cholestasis the common duct was cannulated and drained. The animals were heparinized (3 mg/kg).

Blood flow and blood pressure recording. To measure the hepatic arterial blood flow a wide bore polyethylene tube was inserted into the centrally cut left carotid artery and the blood flow was diverted to a closed perspex optical drop counter filled with silicone oil in turn connected by a short wide bore polyethylene tube to the coeliac artery supplying only the liver and the gallbladder. For simultaneous recording of the overall small intestinal blood supply a similar drop recording unit was utilized connecting the left femoral artery with the centrally cut superior mesenteric artery (Fig. 1). The drop recorder operated ordinate writers in which the ordinates were inversely proportional to the rate of blood flow. The blood flow was recorded on a Grass Polygraph model 7D. For continuous recording of portal pressure a polyethylene tubing was inserted via the splenic vein into the portal vein and connected to a Statham pressure transducer (P23AC) operating in ink-writer. The right femoral artery was connected to another pressure transducer for recording of systemic arterial blood pressure. In control experiments the hepatic arterial perfusion pressure was simultaneously recorded from a T-piece in the tubing connecting the left carotid artery to the coeliac artery close to the point of cannulation of the coeliac artery. No significant difference between this pressure and systemic arterial pressure as recorded from the femoral artery was observed and the

T-piece was therefore not used routinely because of the space limitation in the region.

Nerve stimulation and drug administration. The peripheral nerves along the coeliac artery and the superior mesenteric artery were separated and cut immediately where they emerged from the sympathetic ganglion. The peripheral end of these trunks, containing the postganglionic sympathetic nerve fibres to the liver and small intestine respectively were dissected free and mounted on silver ring electrodes for subsequent selective stimulation. Supramaximal square wave impulses (11 V, 5 ms) were delivered from a Grass stimulator model SEI. The nerves were stimulated at 0.1–16 Hz for 4–10 min. Isoprenaline sulphate dissolved in 0.9% saline was administered in graded close infusions into the hepatic and superior mesenteric arteries by means of an automatic infusion pump at rates which did not exceed 0.4 ml/min.

Calculation of flow. Hepatic arterial vascular resistance was calculated as systemic mean arterial pressure (= hepatic arterial mean perfusion pressure) divided by hepatic arterial blood flow ($\text{ml}/\text{min} \times 100 \text{ g liver}$). Inferior vena cava pressure was in three experiments measured by means of a catheter passed via the right external jugular vein and placed with its tip in the bend of the hepatic vein. The mean pressure varying between 3–5 cm H₂O remained unchanged during the nerve stimulation and was therefore not taken into account in calculation of the hepatic arterial vascular resistance. Superior mesenteric arterial vascular resistance was calculated as the ratio of mean arterial pressure—portal pressure to superior mesenteric blood flow ($\text{ml}/\text{min} \times 100 \text{ g small intestine}$). Resistance is expressed in peripheral resistance units (PRU $100 = 1 \text{ mmHg}/\text{ml}/\text{min} \times 100 \text{ g}$). The weight of the liver and small intestine including the mesenteric lymph nodes was determined after each experiment and the other extirpated organs were also weighed. Values are expressed as means \pm S.E. The significance of differences between sets of data was assessed by Student's *t* test.

RESULTS

1 Basal conditions and infusion of isoprenaline

The resting hepatic arterial blood flow in the acutely denervated liver as determined in 70 cats was $18\text{--}37 \text{ ml}/\text{min} \times 100 \text{ g liver}$ (mean 26.0 ± 2.5), the regional vascular resistance varying between $1.9\text{--}4.8 \text{ PRU}_{100}$ (mean 3.6 ± 0.7). The corresponding values for superior mesenteric flow and resistance were $22\text{--}49 \text{ ml}/\text{min} \times 100 \text{ g intestine}$ (mean 34.0 ± 2.1) and $1.9\text{--}4.6 \text{ PRU}_{100}$ (mean 3.1 ± 0.3) respectively. The blood flow levels at the end of the experiments often lasting for 2–3 h did not differ significantly from these figures neither in the liver nor in the small intestine ($P > 0.1$).

Infusion in the hepatic artery of supramaximal doses of isoprenaline increased hepatic arterial

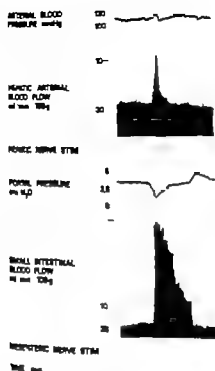


Fig. 4 Cat 3.1 kg. The effects of synchronous stimulation (8 Hz, 5 ms, 11 V) of hepatic and mesenteric nerve stimulation on systemic arterial blood pressure, hepatic arterial blood flow, portal pressure and small intestinal blood flow. Note particularly that the portal pressure returns to the prestimulatory level during the stimulation.

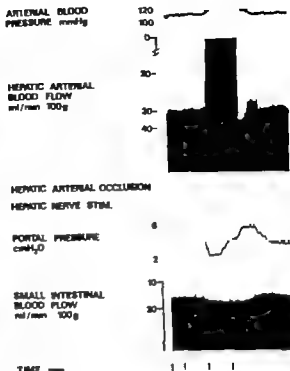


Fig. 5 Cat 2.9 kg. The effects of hepatic nerve stimulation (8 Hz, 5 ms, 12 V) during hepatic arterial occlusion. Note the increase of portal pressure on stimulation during occlusion and the overshoot when the occlusion is released.

markedly during stimulation of the mesenteric nerves. Although this increase was partly due to an increase in mean arterial pressure there was a clear and statistically significant decrease in PRU_{mes} ($P < 0.05$).

C Simultaneous stimulation of the hepatic and mesenteric sympathetic nerves. The effect of simultaneous stimulation of the two vasoconstrictor fibres on hepatic and small intestinal blood flow was largely the same as when the nerve fibres were stimulated separately (Fig. 4). The portal pressure response differed however. Although there was in most experiments an initial pressure reduction, a slight arterial pressure increase amounting to at the most 1.2 cm H₂O was sometimes observed irrespective of the quality and quantity of these initial changes the portal pressure approached the control level on sustained stimulation. Thus, during the steady state the portal pressure did not differ from the prestimulatory level.

III The effects of regional arterial occlusions and sympathetic nerve stimulation

On occlusion of the hepatic artery systemic blood pressure increased with 10–12 mmHg and portal pressure decreased somewhat. This decrease amounting to about 22% of control level was well maintained throughout the occlusion period. The superior mesenteric vascular resistance remained unchanged however. When the hepatic artery was clamped, hepatic nerve stimulation invariably caused an increase in portal pressure. This increase was quantitatively of the same magnitude as that observed during intact hepatic arterial circulation (Fig. 5). On release of the occlusion there was a shortlasting hyperemia and a concomitant further increase of the portal pressure. Occlusion of the superior mesenteric artery increased mean arterial blood pressure to the same extent as did hepatic arterial occlusion. Concomitantly there was an increase in hepatic arterial blood flow of about 8

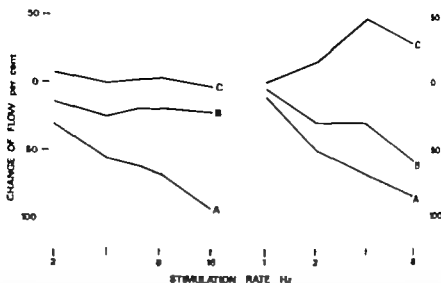


Fig. 3 Frequency response for the hepatic arterial (left panel) and small intestinal blood flow (right panel). The effects of graded vasoconstrictor activation are expressed in per cent of prestimulatory blood flow levels. Note that the peak response (A) is rate dependent in both vascular beds; that the steady state (B) response is rate dependent only in the small intestine and that the immediate post-stimulatory flow (C) the reactive hyperemia (C) is most pronounced in the small intestine.

within 1–2 min a new and well maintained flow level (steady state) was established which was in most experiments slightly below control level. In some animals the blood flow even reached the control level during the stimulation. The blood flow during steady state did not differ significantly at different stimulation frequencies ($P>0.05$). On cessation of stimulation a shortlasting hyperemia (30–60 s) was occasionally seen but was unrelated to the rate of the stimulation.

Corresponding to the peak resistance response there was a considerable but transient rise of the portal pressure which at high frequencies of stimulation amounted to 100–200%. The portal pressure then slowly declined however and after 2–3 min stimulation a steady state level was established at about 30% above the control level. This level was well maintained throughout the stimulation. The differences between the peak response and the steady state level was statistically significant ($P<0.01$). The superior mesenteric arterial blood flow e.g. the portal blood flow increased somewhat. The superior mesenteric vascular resistance remained unchanged ($P>0.2$) however indicating that this blood flow increase was related to the concomitant increase in mean arterial blood pressure regularly observed during the stimulation.

B. The mesenteric sympathetic nerves. Stimulation of the vasoconstrictor fibres to the small intestine evoked the same initial effect on the intestinal blood flow as did hepatic nerve stimulation on the hepatic blood flow. The threshold frequency was in most experiments 0.5 Hz. After an initial peak response the resistance to flow decreased again, and was within 2–3 min followed by a sustained but moderately reduced blood flow (Fig. 2 right panel). The decline towards control level was somewhat slower than that observed during stimulation of the hepatic plexus. As is shown in Fig. 3 right panel the peak response showed a linear correlation to the stimulation frequency ($r=0.68$). Maximum decrease was in most experiments obtained at 8 Hz. In contrast to the observations in the hepatic vascular circuit there was a more pronounced reduction of the blood flow during steady state on high (8 Hz) than on low (2 Hz) stimulation rates ($P<0.001$), and at high rates the initial blood flow reduction in some experiments remained almost unchanged throughout the stimulation. Following cessation, there was a marked hyperemia lasting for several minutes. Simultaneous to the peak resistance response there was a moderate decrease of the portal pressure amounting to about 24%. This initial rapid decrease was followed by a slight increase towards control level. Concomitant to the hyperemia following cessation of stimulation there was a transient increase of the portal pressure amounting to about 18%.

The hepatic arterial blood flow also increased

1967), implying that β -receptor stimulation plays a minor role in the nervous response.

The almost complete recovery of the hepatic arterial blood flow after the initial peak reduction would imply that the hepatic arteries might have a high sensitivity to local metabolic factors. This is also supported by the observation that occlusion of the superior mesenteric artery was followed by a decreased hepatic arterial vascular resistance. It is also possible that the sympathetic nerves have a direct trophic influence on the parenchymal cells of the liver. Redistribution of arterial blood flow which might explain the escape phenomenon in the small intestine (Folkow, Lundgren & Wallentin 1963) appears to be a less probable mechanism in the liver (Greenway & Oshiro 1977).

The marked increase of the portal vein pressure in hepatic sympathetic nerve stimulation has been demonstrated earlier (Greenway et al 1967; Sadig, Jämsä & Esopari 1977; Henrich & Biester 1973; Uchida & Rodbard 1974; Laitinen 1977). The pronounced increase of portal vein pressure obtained on stimulation even when the hepatic arterial supply was occluded speak in favour of a direct vasoconstrictor effect on the portal vascular bed.

Greenway et al. (1967) showed evidence that the portal vein pressure increase was well maintained throughout the stimulation despite the decline of hepatic vascular resistance towards resting values. This is in contrast to the findings in the present study showing a regular decline of the portal pressure on sustained stimulation. Since the decrease appears later than the blood flow increase it can hardly be secondary to changes in the portal blood flow. Such a decline in fact increase as reflected in an increased superior mesenteric flow. Since an increase in portal blood flow would increase portal pressure the decline due to escape from vasoconstrictor fibre influence was even somewhat masked. The explanation for this escape phenomenon is not clear. However, its late appearance as compared with that occurring in the hepatic arterial tract would make accumulation of metabolic factors possible.

It has been previously shown that the reactions in the vascular bed of the stomach and colon to sympathetic nerve action seems to be similar to those observed in the small intestine (Folkow & Laitinen 1971). Thus the tissue volume in all parts of the gastrointestinal tract, as recorded by plethysmographic technique, remains constant throughout

sympathetic vasoconstrictor activation implying that mean hydrostatic capillary pressure is kept unchanged. It has been suggested that such a haemodynamic arrangement serves to protect the gut mucosa from a massive oedema that would inevitably develop if the isovolumetric state was changed into a continuous outward filtration. Considering the big capillary surface of the intestine any increase of resistance in the portal vascular bed during a generalized increase of sympathetic discharge would be potentially deleterious. The result of the present study clearly shows that the portal flow resistance which makes up part of the post capillary resistance for the intestinal vascular bed is not increased when the hepatic vessels are exposed to the same constrictor fibre discharge as the intestinal vessel.

This investigation was supported by grant from the Swedish Medical Research Council (N 17X 3117), from the Faculty of Medicine University of Göteborg. The Swedish Society of Medical Sciences from Göteborgs Läkaresällskap and Avar Gabrielsson Fund.

REFERENCES

- BRAUER, R. W. 1963 Liver circulation and function. *Physiol Rev* 43, 115-215.
- FÄSTH S & HULTÉN L. 1973 The effect of bradykinin on intestinal motility and blood flow. *Acta Chir Scand* 139, 699-705.
- FÄSTH, S. & MARTINSON J. 1973 On the possible role of bradykinin in functional hyperemia of cat stomach. *Acta Physiol Scand* 89, 334-341.
- FOLKOW B & NEIL, E. 1971 *Circulation*. Oxford University Press, London.
- FOLKOW B, LUNDGREN B & WALLENTIN L. 1963 Studies on the relationship between flow resistance, capillary filtration coefficient and regional blood volume in the intestine of the cat. *Acta Physiol Scand* 57, 270-283.
- GREENWAY C. V., LAWSON A. E. & MELLANDER, S. 1967 The effect of stimulation of the hepatic nerves, infusions of noradrenaline and occlusion of the carotid arteries on liver blood flow in the anaesthetized cat. *J Physiol (Lond.)* 192, 21-41.
- GREENWAY C. V. & OSHIRO D. 1972 Comparison of the effects of hepatic nerve stimulation on arterial flow, distribution of arterial and portal flows and blood content in the livers of anaesthetized cat and dog. *J Physiol (Lond.)* 227, 487-501.
- GREENWAY C. V. & STARK R. D. 1971 Hepatic vascular bed. *Physiol Rev* 51, 23-63.
- HENRICH H & BIESTER, J. 1973 Localization of the vascular escape phenomenon in the intestinal circulation. *Pflügers Arch Ges Physiol* 332, Suppl. 60-62, R 6.

ml/min \times 100 g corresponding to a 30% increase PRU decreased 70% indicating that this increase in hepatic arterial blood flow was partly caused by a decrease of hepatic vascular resistance. After cessation of the occlusion there was a profound hyperemia in the small intestinal vascular bed lasting for about 2 min. On superior mesenteric artery occlusion the decrease in portal pressure was marked amounting to about 70%. Hepatic nerve stimulation during occlusion caused a slight increase of portal pressure amounting to at the most 1 cm H₂O. This was not the case when the mesenteric nerves were stimulated. Simultaneous occlusion of the hepatic and superior mesenteric arteries lowered portal pressure to 0.4–0.7 cm H₂O. When both these arterial supplies were clamped hepatic nerve stimulation increased portal pressure with about 0.5 cm H₂O. On release of the occlusion a marked hyperemia was observed in both vascular beds. The post occlusion hyperemia was of the same magnitude and duration as that observed after separate occlusion of the circuits.

DISCUSSION

The present observation that occlusion of the hepatic artery does not affect portal blood flow is consistent with previous findings (Kock et al 1977, Krarup & Larsen 1974). During hepatic artery occlusion there was a well maintained reduction of portal pressure amounting to about 70% compared to a reduction of about 70% when the superior mesenteric artery was clamped. These findings would imply that small intestinal blood flow contributes more to maintenance of the portal pressure than the hepatic arterial blood flow, but also that there might exist an efficient pressure reduction between the high and low pressure systems. It might even be so that the reduction of portal pressure obtained during hepatic artery occlusion is not entirely caused by a direct influence of hepatic arterial pressure on the portal vascular bed but might at least partly be due to a decreased tone in presinusoidal sphincters.

Occlusion of mesenteric blood flow however caused a significant increase in hepatic arterial flow partly caused by a decrease in hepatic vascular resistance. This decrease in resistance might probably be due to hepatic hypoxia as has been suggested by Kock et al (1972). Occlusion of both

vascular supplies lowered portal pressure to the "critical closing pressure" of the postsinusoidal veins. In the present study this pressure was lower than that reported in studies on dogs, however (Mitzner 1974). This difference might be explained by the fact that canine postsinusoidal veins have a more pronounced potential for sphincteric action than those of the cat (Ungváry & Donath 1969).

Vasoconstrictor fiber stimulation caused an initial peak blood flow reduction in both vascular beds. This peak response correlated in a linear fashion to the logarithm of the stimulation rate. Despite continuous stimulation the peak resistance response was transient and followed by a second phase of less pronounced vasoconstriction. The escape phenomenon differed in some important respects in the two vascular circuits, however. Thus the hepatic vascular resistance regularly subsided within 1–2 min and hepatic arterial blood flow reached a new steady state which was only about 70% below the control blood flow, irrespective of stimulation frequency. By contrast the peak resistance response in the small intestine was more prolonged and a steady state level was as a rule not attained until 2–3 min. The escape phenomenon was not observed in all animals, an observation which is in accordance with earlier findings (Fellow & Neil 1971). Moreover in contrast to what was observed in the hepatic vascular bed the magnitude of the "steady state" blood flow was dependent of the stimulation rates, being lower at high discharge rates. Moreover poststimulatory hyperemia was more pronounced and regular in the small intestine than in the liver.

The mechanisms responsible for the autoregulatory escape are still obscure. In the intestine this escape phenomenon has been demonstrated both in the cat in the dog (Greenway & Oshiro 1972) and in man (Huhtén, Lindhagen & Lundgren 1977) but appears to be absent in the liver of the dog (Greenway & Oshiro 1972, Richardson & Withrington 1977a). A similar phasic change of hepatic and intestinal blood flow has been described during infusion of noradrenaline however (Greenway et al 1967, Ross 1971, Schekadeh, Price & Jacobson 1969, Richardson & Withrington 1977b).

Under these experimental conditions the escape was greatly attenuated by propranolol in contrast to the response elicited by nerve stimulation which was largely unaffected (Greenway et al

1967), implying that β -receptor stimulation plays a minor role in the nervous response.

The almost complete recovery of the hepatic arterial blood flow after the initial peak reduction, could imply that the hepatic arteries might have a high sensitivity to local metabolic factors. This is also supported by the observation that occlusion of the superior mesenteric artery was followed by a decreased hepatic arterial vascular resistance. It is also possible that the sympathetic nerves have a direct trophic influence on the parenchymal cells of the liver. Redistribution of arterial blood flow which might explain the escape phenomenon in the small intestine (Folkow, Lundgren & Wallentin 1963) appears to be a less probable mechanism in the liver (Greenway & Oshiro 1977).

The marked increase of the portal vein pressure in hepatic sympathetic nerve stimulation has been demonstrated earlier (Greenway et al 1967, Sædigh, Jønkær & Enquist 1972, Heinrich & Biester 1973, Gluick & Rodbard 1974, Lærvi 1977). The pronounced increase of portal vein pressure obtained in this study even when the hepatic arterial supply was occluded speaks in favour of a direct vasoconstrictor effect on the portal vascular bed.

Greenway et al. (1967) showed evidence that the portal vein pressure increase was well maintained throughout the stimulation despite the decline of hepatic vascular resistance towards resting values. This is in contrast to the findings in the present study showing a regular decline of the portal pressure on sustained stimulation. Since the decrease appears later than the blood flow increase it can probably be secondary to changes in the portal blood flow which did in fact increase as reflected in an increased superior mesenteric flow. Since an increase in portal blood flow would increase portal pressure the decline due to escape from vasoconstrictor fibre influence was even somewhat delayed. The explanation for this escape phenomenon is not clear. However its late appearance as compared with that occurring in the hepatic arterial circuit would make accumulation of metabolic factors possible.

It has been previously shown that the reactions in the vascular bed of the stomach and colon to sympathetic nerve activation seems to be similar to those observed in the small intestine (Folkow & Lærvi 1977). Thus the tissue volume in all parts of the gastrointestinal tract, as recorded by plethysmographic technique remains constant throughout

sympathetic vasoconstrictor activation implying that mean hydrostatic capillary pressure is kept unchanged. It has been suggested that such a haemodynamic arrangement serves to protect the gut mucosa from a massive oedema that would inevitably develop if the isovolumetric state was changed into a continuous outward filtration. Considering the big capillary surface of the intestine any increase of resistance in the portal vascular bed during a generalized increase of sympathetic discharge would be potentially deleterious. The result of the present study clearly shows that the portal flow resistance which makes up part of the post capillary resistance for the intestinal vascular bed is not increased when the hepatic vessels are exposed to the same constrictor fibre discharge as the intestinal vessel.

This investigation was supported by grant from the Swedish Medical Research Council (No. 17X 3117), from the Faculty of Medicine University of Göteborg, The Swedish Society of Medical Sciences from Göteborgs Läkaresällskap and Assar Gabrielsson's Fund.

REFERENCES

- BRÄUER R. W. 1963 Liver circulation and function. *Physiol Rev* 43 115-13.
- FÄSTH S. & HULTÉN L. 1973 The effect of bradykinin on intestinal motility and blood flow. *Acta Chir Scand* 139 699-705.
- FÄSTH S. & MARTINSON J. 1973. On the possible role of bradykinin in functional hyperemia of cat's stomach. *Acta Physiol Scand* 89: 334-341.
- FOLKOW B. & NELL, E. 1971 Circulation. Oxford University Press, London.
- FOLKOW B., LUNDGREN O. & WALLENTIN I. 1963 Studies on the relationship between flow resistance, capillary filtration coefficient and regional blood volume in the intestine of the cat. *Acta Physiol Scand* 57 270-283.
- GREENWAY C. V., LAWSON A. E. & MELLANDER, S. 1967 The effects of stimulation of the hepatic nerves, infusions of noradrenaline and occlusion of the carotid arteries on liver blood flow in the anesthetized cat. *J Physiol (Lond)* 192: 21-41.
- GREENWAY C. V. & OSHIRO O. 1972. Comparison of the effects of hepatic nerve stimulation on arterial flow distribution of arterial and portal flow and blood content in the livers of anesthetized cats and dogs. *J Physiol (Lond)* 227 487-501.
- GREENWAY C. V. & STARK R. 1971 Hepatic vascular bed. *Physiol Rev* 51 23-65.
- HENRICH H. & BIESTER, J. 1973 Localization of the vascular escape phenomenon in the intestinal circulation. *Pflügers Arch Ges Physiol* 332 Suppl 60-62, R 6...

ml/min \times 100 g corresponding to a 30% increase PRU decreased 30% indicating that this increase in hepatic arterial blood flow was partly caused by a decrease of hepatic vascular resistance. After cessation of the occlusion there was a profound hyperemia in the small intestinal vascular bed lasting for about 2 min. On superior mesenteric artery occlusion the decrease in portal pressure was marked amounting to about 70%. Hepatic nerve stimulation during occlusion caused a slight increase of portal pressure amounting to at the most 1 cm H₂O. This was not the case when the mesenteric nerves were stimulated. Simultaneous occlusion of the hepatic and superior mesenteric arteries lowered portal pressure to 0.4–0.7 cm H₂O. When both these arterial supplies were clamped hepatic nerve stimulation increased portal pressure with about 0.5 cm H₂O. On release of the occlusion a marked hyperemia was observed in both vascular beds. The post occlusion hyperemia was of the same magnitude and duration as that observed after separate occlusion of the circuits.

DISCUSSION

The present observation that occlusion of the hepatic artery does not affect portal blood flow, is consistent with previous findings (Kock et al. 1972; Krarup & Larsen 1974). During hepatic artery occlusion there was a well maintained reduction of portal pressure amounting to about 70% compared to a reduction of about 70% when the superior mesenteric artery was clamped. These findings would imply that small intestinal blood flow contributes more to maintenance of the portal pressure than the hepatic arterial blood flow, but also that there might exist an efficient pressure reduction between the high and low pressure systems. It might even be so that the reduction of portal pressure obtained during hepatic artery occlusion is not entirely caused by a direct influence of hepatic arterial pressure on the portal vascular bed but might at least partly be due to a decreased tone in presinusoidal sphincters.

Occlusion of mesenteric blood flow however caused a significant increase in hepatic arterial flow partly caused by a decrease in hepatic vascular resistance. This decrease in resistance might probably be due to hepatic hypoxia as has been suggested by Kock et al. (1972). Occlusion of both

vascular supplies lowered portal pressure to the critical closing pressure of the postsinusoidal veins. In the present study this pressure was lower than that reported in studies on dogs, however (Mitzner 1974). This difference might be explained by the fact that canine postsinusoidal veins have a more pronounced potential for sphincteric action than those of the cat (Ungváry & Donath 1969).

Vasoconstrictor fiber stimulation caused an initial peak blood flow reduction in both vascular beds. This peak response correlated in a linear fashion to the logarithm of the stimulation rate. Despite continuous stimulation the peak resistance response was transient and followed by a second phase of less pronounced vasoconstriction. The "escape phenomenon" differed in some important respects in the two vascular circuits, however. Thus the hepatic vascular resistance regularly subsided within 1–2 min and hepatic arterial blood flow reached a new steady state which was only about 30% below the control blood flow, irrespective of stimulation frequency. By contrast the peak resistance response in the small intestine was more prolonged and a steady state level was as a rule not attained until 2–3 min. The escape phenomenon was not observed in all animals, an observation which is in accordance with earlier findings (Fellow & Neil 1971). Moreover in contrast to what was observed in the hepatic vascular bed the magnitude of the steady state blood flow was dependent of the stimulation rates being lower at high discharge rates. Moreover poststimulatory hyperemia was more pronounced and regular in the small intestine than in the liver.

The mechanisms responsible for the autoregulatory escape are still obscure. In the intestine this escape phenomenon has been demonstrated both in the cat in the dog (Greenway & Oshiro 1972) and in man (Hultén, Lindhagen & Lundgren 1977) but appears to be absent in the liver of the dog (Greenway & Oshiro 1972; Richardson & Withrington 1977a). A similar phasic change of hepatic and intestinal blood flow has been described during infusion of noradrenaline however (Greenway et al. 1967; Ross 1971; Scheludeh, Price & Jacobson 1969; Richardson & Withrington 1977b).

Under these experimental conditions the "escape" was greatly attenuated by propranolol in contrast to the response elicited by nerve stimulation which was largely unaffected (Greenway et al.

Blood flow changes in the duck during thermal panting

LAUS BECH and KJELL JOHANSEN

Department of Zoophysiology University of Aarhus, Denmark

BECH L. & JOHANSEN K. Blood flow changes in the duck during thermal panting. *Acta Physiol Scand* 1980, 110: 351-355. Received 14 Febr. 1980. ISSN 0001-6772. Department of Zoophysiology University of Aarhus, Denmark.

Carotid and sciatic blood flow have been measured in resting and panting Pekin ducks using electromagnetic flowmeters. Panting induced by high ambient temperatures caused the carotid blood flow to increase from 9.6 to 76.0 ml min⁻¹ while the sciatic flow decreased slightly from 41.6 to 38.1 ml min⁻¹. During panting breathing rate increased 10-20 times but there were no significant changes in heart rate and mean arterial blood pressure. The carotid peripheral resistance was therefore greatly reduced, whereas sciatic resistance remained unchanged or increased slightly. The vascular beds perfused by the sciatic (legs) and carotid (upper respiratory tract) arteries are both important for heat dissipation. This study shows that when heat dissipation from the naked legs becomes inefficient due to high ambient temperature, blood flow in the sciatic tended to decline while conversely panting as associated with large increase in carotid flow.

Key words. Hyperthermia, heat dissipation, panting, Pekin duck, regional blood flow

Acting sweat glands birds must depend on heat dissipation by evaporation from the respiratory tract. As increasing environmental temperatures tend to reverse the skin to ambient temperature gradient. Typically this respiratory heat loss by evaporation is greatly increased with onset of panting (Richards 1970). Optimising heat dissipation during panting depends on 3 main factors: (1) The increased ventilation associated with the 10-20 increase in breathing rate; (2) Passage of exhaled air across the largest possible surface area of the upper respiratory tract; and (3) An increase in blood flow to the evaporating surfaces conveying heat from the body core to the sites of heat dissipation.

The magnitude and pattern of the ventilatory changes during panting have been well studied for many species (Richard 1970; Laskowski 1972). The nature of blood flow changes to evaporating surfaces in panting birds has, however, to our knowledge only been studied by estimation of changes in regional blood flow distribution based on injection of radioactive microspheres (Wolfe *et al.* 1978a, b).

The present study seeks to establish the changes in blood flow to and vascular resistance in the head

and legs of domestic ducks associated with hyperthermia and panting.

MATERIALS AND METHODS

Domestic ducks were obtained from local supplier. They were housed indoors at room temperatures (20-22°C) and exposed to a 10 to 14 h light-dark cycle. They were fed commercial duck pellets and had access to water at all times.

Surgery for blood vessel catheterizations and electromagnetic blood flow probe placements were done during general anaesthesia (equithal, 2.5 ml/kg administered *sc.*). The carotid arteries were exposed at the lower ventral side of the neck near the thoracic inlet. A Starbarn 0.0 mm electromagnetic flow probe was placed around the left carotid artery and anchored in place by means of stabilizing plastic rod attached to the flow probe and sutured to the neck musculature running parallel to the artery. The leads were extended out through small skin incisions and secured by sutures. Using a similar procedure 0.0 mm flow probe was also placed around the left sciatic artery. This artery was exposed by dissection through the M. iliotibialis. A maximum of two days were allowed for post operative recovery before experimentation was started.

Calibration of the flow probes were done *in situ*. After termination of experiments the ducks were given large dose of heparin and killed with an overdose of anaesthesia. The left carotid artery as well as the left sci-

- HULTÉN L. 1969 Extrinsic nervous control of colonic motility and blood flow. *Acta Physiol Scand* Suppl 335
- HULTÉN L., LINDHAGEN J. & LUNDGREN O. 1977 The sympathetic nervous control of intramural blood flow in the feline and human intestine. *Gastroenterology* 72: 41-48
- KIKUCHI Y. & ROBBARD E. 1974 Autoregulatory escape and post-escape hyperaemia in a capillary model. *Cardiovasc Res* 8: 395-403
- KOCK N. O., HAHNLOSER P., RÖDING H. & WORTHINGTON S. 1977 Interaction between portal venous and hepatic arterial blood flow. An experimental study in the dog. *Surgery* 72: 414-419
- KRÄRUP N. & LARSEN J. 1974 The immediate effects of ligation of the hepatic artery on liver hemodynamics and liver function in the cat. *Acta Physiol Scand* 91: 441-446
- LAUTT W. W. 1977 Effect of stimulation of hepatic nerves on hepatic O_2 uptake and blood flow. *Amer J Physiol* 233: G55-G56
- MARTINSON J. 1965 Studies on the efferent vagal control of the stomach. *Acta Physiol Scand* 65 Suppl. 55
- MITZNER, W. 1974 Hepatic outflow resistance, sinusoidal pressure and the vascular waterfall. *Amer J Physiol* 227: 513-519
- RICHARDSON P. D. J. & WITHERINGTON P. G. 1977a Glucagon inhibition of hepatic arterial responses to hepatic nerve stimulation. *Amer J Physiol* 233: 647-654
- RICHARDSON P. D. J. & WITHERINGTON P. G. 1977b The role of β -adrenoceptors in the responses of the hepatic arterial vascular bed of the dog to picroleptophrine, isoprenaline, noradrenaline and adrenaline. *Brit J Pharmacol* 60: 39-49
- ROSS G. 1971 Escape of mesenteric vessels from α_1 adrenergic and nonadrenergic vasoconstriction. *Amer J Physiol* 221: 1217-1222
- SADIQ S., SUDHAKAR P. & ENQUIST P. 1977 Hepatic congestion and bile secretion. *Arch Surg* 111: 749-751
- SHEHADER Z., PRICE, W. & JACOBSON E. 1969 Effects of vasoactive agents on intestinal blood flow and motility in the dog. *Amer J Physiol* 16: 386-397
- UNGVARY G. Y. & DONÁTI T. 1969 On the mesenteric sympathetic innervation of the liver. *Acta Anat* 7: 446-459
- WALLENTIN I. 1966 Studies on intestinal circulation. Thesis. *Acta Physiol Scand* 69 Suppl. 779

Blood flow changes in the duck during thermal panting

LAUS BECH and KJELL JOHANSEN

Department of Zoophysiology, University of Aarhus, Denmark

BECH C & JOHANSEN K., Blood flow changes in the duck during thermal panting. *Acta Physiol Scand* 1980; 110: 351-355. Received 14 Febr. 1980. ISSN 0001-6772. Department of Zoophysiology, University of Aarhus, Denmark.

Carotid and aortic blood flow have been measured in resting and panting Pekin ducks using electromagnetic flowmeters. Panting induced by high ambient temperature caused the carotid blood flow to increase from 9.6 to 76.0 ml min⁻¹ while the aortic flow declined slightly from 41.8 to 38.1 ml min⁻¹. During panting breathing rate increased 10-20 times but there were no significant changes in heart rate and mean arterial blood pressure. The carotid peripheral resistance was therefore greatly reduced whereas aortic resistance remained unchanged or increased slightly. The vascular bed perfused by the aortic (legs) and carotid (upper respiratory tract) arteries are both important for heat dissipation. This study shows that heat dissipation from the naked legs becomes inefficient due to high ambient temperature; blood flow in the aorta tended to decline while conversely panting is associated with large increase in carotid flow.

Key words: Hyperthermia, heat dissipation, panting, Pekin duck, regional blood flow.

Acting sweat glands birds must depend on heat loss by evaporation from the respiratory tract when increasing environmental temperatures inhibit or reverse the skin to ambient temperature balance. Typically this respiratory heat loss by evaporation is greatly increased with onset of panting (Richards 1970). Optimising heat dissipation during panting depends on 3 main factors: (1) The increased ventilation associated with the 10-20% increase in breathing rate; (2) Passage of exhaled air across the largest possible surface area of the upper respiratory tract; and (3) An increase in blood flow to the evaporating surfaces conveying heat from the body core to the sites of heat dissipation.

The magnitude and pattern of the ventilatory changes during panting have been well studied for many species (Richards 1970, Lasiewski 1977). The nature of blood flow changes to evaporating surfaces in panting birds has, however, to our knowledge, only been studied by estimation of changes in regional blood flow distribution based on injection of radioactive microspheres (Wolfenson et al. 1978, b).

The present study seeks to establish the changes in blood flow to and vascular resistance in the head

and legs of domestic ducks associated with hyperthermia and panting.

MATERIALS AND METHODS

Domestic ducks were obtained from a local supplier. They were housed indoors at room temperatures (20-22°C) and exposed to a 10 to 14 h light-dark cycle. They were fed commercial duck pills and had access to water at all times.

Surgery for blood vessel catheterizations and electromagnetic blood flow probe placements were done during general anaesthesia (equithal, 2.5 ml/kg administered i.v.). The carotid arteries were exposed at the lower ventral side of the neck near the thoracic inlet. A Statathon 2.0 mm electromagnetic flow probe was placed around the left carotid artery and anchored in place by means of a stabilizing plastic rod attached to the flow probe and sutured to the neck musculature running parallel to the artery. The leads were extended out through a small skin incision and secured by sutures. Using similar procedure 2.0 mm flow probe was also placed around the left sciatic artery. This artery was exposed by dissection through the M. iliothoracicus. A minimum of two days were allowed for post operative recovery before experimentation was started.

Calibration of the flow probes were done *in situ*. After termination of experiments the ducks were given a large dose of heparin and killed with an overdose of anaesthesia. The left carotid artery as well as the left sci-

- HULTÉN L. 1969 Extrinsic nervous control of colonic motility and blood flow. *Acta Physiol Scand* Suppl 335
- HULTÉN L., LINDHAGEN J. & LUNDGREN D. 1977 The sympathetic nervous control of intramural blood flow in the feline and human intestine. *Gastroenterology* 72: 41-48
- KIKUCHI Y. & ROXBARD S. 1974 Autoregulatory escape and post-escape hyperaemia in a capillary model. *Cardiovasc Res* 8: 395-403
- KOCK N. G., HAHNLOSER P., RODING B. & WORTHINGTON D. 1972 Interaction between portal venous and hepatic arterial blood flow. An experimental study in the dog. *Surgery* 72: 414-419
- KRÄRUP N. & LARSEN J. 1974 The immediate effects of ligation of the hepatic artery on liver hemodynamics and liver function in the cat. *Acta Physiol Scand* 91: 441-446
- LAUTT W. W. 1977 Effect of stimulation of hepatic nerves on hepatic O_2 uptake and blood flow. *Amer J Physiol* 233: 652-656
- MARTINSON J. 1965 Studies on the efferent vagal control of the stomach. *Acta Physiol Scand* 65 Suppl 35
- MITZNER W. 1974 Hepatic outflow resistance, sinusoid pressure and the vascular waterfall. *Amer J Physiol* 227: 513-519
- RICHARDSON P. D. J. & WITHERINGTON P. G. 1977a Glucagon inhibition of hepatic arterial responses to hepatic nerve stimulation. *Amer J Physiol* 233: 647-654
- RICHARDSON P. D. J. & WITHERINGTON P. G. 1977b The role of β -adrenoreceptors in the responses of the hepatic arterial vascular bed of the dog to phenylephrine, isoprenaline, noradrenaline and adrenaline. *Brit J Pharmacol* 60: 39-49
- ROSS G. 1971 Escape of mesenteric vessels from adrenergic and nonadrenergic vasoconstriction. *Amer J Physiol* 221: 117-122
- SADIQ S., SUDHAKAR P. & ENQUIST P. W. 1971 Hepatic congestion and bile secretion. *Arch Surg* 106: 749-751
- SHEHADEH Z., PRICE W. & JACOBSON E. 1968 Effects of vasoactive agents on intestinal blood flow and motility in the dog. *Amer J Physiol* 216: 386-392
- UNGVARY G. Y. & DONÁTH T. 1969 On the monoaminergic innervation of the liver. *Acta Anat* 72: 446-459
- WALLENTIN I. 1966. Studies on intestinal circulation. Thesis. *Acta Physiol Scand* 69 Suppl 79

Blood flow changes in the duck during thermal panting

LAUS BECH and KJELL JOHANSEN

Department of Zoophysiology, University of Aarhus, Denmark

BECH C. & JOHANSEN K. Blood flow changes in the duck during thermal panting. *Acta Physiol Scand* 1980, 110: 351-355. Received 14 Febr. 1980. ISSN 0001-6772. Department of Zoophysiology, University of Aarhus, Denmark.

Carotid and sciatic blood flow have been measured in resting and panting Pekin ducks using electromagnetic flow meters. Panting induced by high ambient temperature caused the carotid blood flow to increase from 9.6 to 26.0 ml min⁻¹ while the sciatic flow declined slightly from 41.6 to 38.1 ml min⁻¹. During panting breathing rate increased 10-20 times but there were no significant changes in heart rate and mean arterial blood pressure. The carotid peripheral resistance was therefore greatly reduced whereas sciatic resistance remained unchanged or increased slightly. The vascular bed perfused by the sciatic (legs) and carotid (upper respiratory tract) arteries are both important for heat dissipation. This study shows that when heat dissipation from the naked legs becomes inefficient due to high ambient temperature, blood flow in the sciatic tended to decline while conversely panting was associated with a large increase in carotid flow.

Key words. Hyperthermia, heat dissipation, panting, Pekin duck, regional blood flow

Among sweat glands birds must depend on heat dissipation by evaporation from the respiratory tract when increasing environmental temperatures inhibit or reverse the skin to ambient temperature gradient. Typically this respiratory heat loss by evaporation is greatly increased with onset of panting (Richards 1970). Optimizing heat dissipation during panting depends on 3 main factors: (1) The increased ventilation associated with the 10-20% increase in breathing rate, (2) Passage of exhaled air across the largest possible surface area of the upper respiratory tract, and (3) An increase in blood flow to the evaporating surfaces conveying heat from the body core to the sites of heat dissipation.

The magnitude and pattern of the ventilatory changes during panting have been well studied for many species (Richards 1970; Linsley 1977). The nature of blood flow changes to evaporating surfaces in panting birds has, however, to our knowledge only been studied by estimation of changes in regional blood flow distribution based on injection of radioactive microspheres (Wolffensieck *et al.* 1978a, b).

The present study seeks to establish the changes in blood flow to and vascular resistance in the head

and legs of domestic ducks associated with hyperthermia and panting.

MATERIALS AND METHODS

Domestic ducks were obtained from a local supplier. They were housed indoors at room temperatures (20-22°C) and exposed to a 10 to 14 h light-dark cycle. They were fed commercial duck pellets and had access to water at all times.

Surgery for blood vessel catheterization and electromagnetic blood flow probe placements were done during general anaesthesia (equithal 2.5 ml kg⁻¹ administered i.m.). The carotid arteries were exposed at the lower ventral side of the neck near the thoracic inlet. A Statkon 2.0 mm electromagnetic flow probe was placed around the left carotid artery and anchored in place by means of a stabilizing plastic rod attached to the flow probe and sutured to the neck musculature running parallel to the artery. The leads were extended out through a small skin incision and secured by sutures. Using similar procedures a 0 mm flow probe was also placed around the left sciatic artery. This artery was exposed by dissection through the M. iliothibialis. A minimum of two days were allowed for post operative recovery before experimentation was started.

Calibration of the flow probes were done *in situ*. After termination of experiments the ducks were given a large dose of heparin and killed with an overdose of anaesthesia. The left carotid artery as well as the left sci-

- HULTÉN L. 1969 Extrinsic nervous control of colonic motility and blood flow. *Acta Physiol Scand Suppl* 335.
- HULTÉN L., LINDHAGEN J. & LUNDGREN B. 1977 The sympathetic nervous control of intramural blood flow in the feline and human intestine. *Gastroenterology* 72, 41-48.
- KIKUCHI Y. & ROXBARD S. 1974 Autoregulatory escape and post-escape hyperaemia in a capillary model. *Cardiovasc Res* 8, 395-403.
- KOCK N. & HAHNLOSER P., RODING B. & WORTHINGTON S. 1972. Interaction between portal venous and hepatic arterial blood flow. An experimental study in the dog. *Surgery* 72, 414-419.
- KRARUP N. & LARSEN J. 1974 The immediate effects of ligation of the hepatic artery on liver hemodynamics and liver function in the cat. *Acta Physiol Scand* 91, 441-446.
- LAUTT W. W. 1977 Effect of stimulation of hepatic nerves on hepatic O_2 uptake and blood flow. *Amer J Physiol* 232, 657-656.
- MARTINSON J. 1965 Studies on the efferent vagal control of the stomach. *Acta Physiol Scand* 65 Suppl. 59.
- MITZNER W. 1974 Hepatic outflow resistance, sinusoid pressure and the vascular waterfall. *Amer J Physiol* 227, 513-519.
- RICHARDSON P. D. J. & WITHERINGTON P. G. 1977a. Olfacagon inhibition of hepatic arterial responses to hepatic nerve stimulation. *Amer J Physiol* 233, 647-654.
- RICHARDSON P. D. J. & WITHERINGTON P. G. 1977b. The role of β -adrenoreceptors in the response of the hepatic arterial vascular bed of the dog to phenylephrine, isoprenaline, noradrenaline and adrenaline. *Brit J Pharmacol* 60, 39-49.
- ROSS G. 1971. Escape of mesenteric vessels from adrenergic and nonadrenergic vasoconstriction. *Amer J Physiol* 221, 1217-1222.
- SADIQ S. & SUDHAKAR P. & ENQUIST P. 1977. Hepatic congestion and bile secretion. *Arch Surg* 107, 749-751.
- SHEHADEH Z., PRICE W. & JACOBSON E. 1968. Effects of vasoactive agents on intestinal blood flow and motility in the dog. *Amer J Physiol* 216, 386-392.
- UNGVARY G. Y. & DONÁTHI T. 1969. On the monoaminergic innervation of the liver. *Acta Anat* 72, 446-459.
- WALLENTIN I. 1966. Studies on intestinal circulation. Thesis. *Acta Physiol Scand* 69 Suppl. 79.

Blood flow changes in the duck during thermal panting

LAUS BECH and KJELL JOHANSEN

Department of Zoophysiology, University of Aarhus, Denmark

BECH C & JOHANSEN K. Blood flow changes in the duck during thermal panting. *Acta Physiol Scand* 1980; 110: 331-335. Received 14 Febr. 1980. ISSN 0001-6772. Department of Zoophysiology, University of Aarhus, Denmark.

Carotid and sciatic blood flow have been measured in resting and panting Pekin ducks using electromagnetic flowmeters. Panting induced by high ambient temperature caused the carotid blood flow to increase from 9.6 to 26.0 ml min⁻¹ while the sciatic flow declined slightly from 41.6 to 38.1 ml min⁻¹. During panting breathing rate increased 10-20 times but there were no significant changes in heart rate and mean arterial blood pressure. The carotid peripheral resistance was therefore greatly reduced whereas sciatic resistance remained unchanged or increased slightly. The vascular beds perfused by the sciatic (legs) and carotid (upper respiratory tract) arteries are both important for heat dissipation. This study shows that when heat dissipation from the naked legs becomes inefficient due to high ambient temperature, blood flow in the sciatic tended to decline while conversely panting was associated with a large increase in carotid flow.

Key words: Hyperthermia, heat dissipation, panting, Pekin duck, regional blood flow

Among recent glassed birds must depend on heat loss by evaporation from the respiratory tract when increasing environmental temperatures inhibit or reverse the skin to ambient temperature gradient. Typically this respiratory heat loss by evaporation is greatly increased with onset of panting (Richards 1970). Optimising heat dissipation during panting depends on 3 main factors: (1) The increased ventilation associated with the 10-20% increase in breathing rate; (2) Passage of cooled air across the largest possible surface area of the upper respiratory tract; and (3) A decrease in blood flow to the evaporating surfaces conveying heat from the body core to the sites of heat dissipation.

The magnitude and pattern of the ventilatory changes during panting have been well studied for many species (Richards 1970; Luskowski 1972). The nature of blood flow changes to evaporating surfaces in panting birds has, however, to our knowledge only been studied by estimation of changes in regional blood flow distribution based on injection of radioactive microspheres (Wolfenson et al. 1978, b).

The present study seeks to establish the changes in blood flow to and vascular resistance in the head

and legs of domestic ducks associated with hyperthermia and panting.

MATERIALS AND METHODS

Domestic ducks were obtained from a local supplier. They were housed indoors at room temperatures (20-22°C) and exposed to 10 to 14 h light-dark cycle. They were fed commercial duck pellets and had access to water at all times.

Surgery for blood vessel catheterizations and electromagnetic blood flow probe placement were done during general anaesthesia (equithal, 0.5 ml/kg administered i.v.). The carotid arteries were exposed at the lower ventral side of the neck near the thoracic inlet. A Statham 2.0 mm electromagnetic flow probe was placed around the left carotid artery and anchored in place by means of a stabilizing plastic rod attached to the flow probe and sutured to the neck musculature running parallel to the artery. The leads were extended out through a small skin incision and secured by sutures. Using similar procedures a 0.8 mm flow probe was also placed around the left sciatic artery. This artery was exposed by dissection through the M. abductor. A minimum of 14 days were allowed for post-operative recovery before experimentation was started.

Calibration of the flow probes were done in situ. After termination of experiments the ducks were given a large dose of heparin and killed with an overdose of anaesthesia. The left carotid artery as well as the left sci-

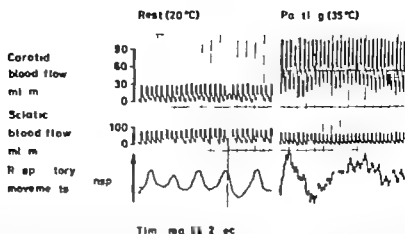


Fig. 1 Recordings of unilateral carotid and sciatic blood flow measured at thermoneutral condition (ambient temperature 20°C) and after 1 hour continued panting at ambient temperature of 35°C. Also shown are the breathing movements depicting the increase in respiratory frequency during panting.

atic artery were cannulated on both sides of the flow probes with polyethylene tubings (PE 60). Subsequently the arteries were perfused with a physiological salt solution using a Harvard peristaltic pump calibrated volumetrically. In this way the flow probes were calibrated in the same position as during the experiments.

Blood flow was measured by Statham electromagnetic flow meters (model SP-202) and mean and pulsatile values recorded on a 6-channel Brush recorder (Mark 60).

Catheters for blood pressure recording were implanted via the brachial artery. The catheter was flushed daily with heparinized saline. Arterial blood pressures were measured using Statham pressure transducers (model P23d). The pressures were recorded on the 6-channel Brush recorder.

Cloacal and ambient temperatures were recorded by an Ellab potentiometer and displayed on a Beckman recorder (model 511A) together with the output from a mercury strain gauge placed around the chest of the ducks and used for monitoring breathing rate.

During experimentation the ducks were placed in a

small box which prevented movement but allowed normal standing and sitting postures. The box was placed in a climatic chamber where the temperature and humidity could be controlled to within $\pm 0.5^\circ\text{C}$ and 5% respectively. Following 3 hours rest at 20°C and relative humidity 50% measurements of all parameters were obtained and the climatic chamber temperature was set at 35°C while retaining the relative humidity at 50%. The bird started panting 45–60 minutes after resetting of the temperature. Following one hour of continued panting all parameters were measured again. Only measurements from periods when the birds were resting have been used in the analyses.

RESULTS

Fig. 1 shows records of unilateral carotid and sciatic blood flow in a resting duck in thermoneutral conditions (20°C) and following 1 h of sustained panting at an ambient temperature of 35°C. Respiratory movements giving frequency of breathing are

Table 1 Comparison between body temperature, breathing rate and circulatory variables in Pekin ducks at rest and during panting

Mean values \pm S.E. N.S. = not significant

		Resting (20°C)	Panting (35°C)	P
Body temperature ($^\circ\text{C}$)	9	41.3 \pm 0.4	41.8 \pm 0.5	<0.05
Breathing rate (min ⁻¹)	9	18.7 \pm 6.1	35 \pm 7.8	<0.001
Unilateral carotid blood flow (ml min ⁻¹)	9	9.3 \pm 3	26.0 \pm 9.0	<0.001
Unilateral sciatic blood flow (ml min ⁻¹)	5	41.6 \pm 4.3	38.1 \pm 4.9	N.S.
Heart rate (min ⁻¹)	9	146.6 \pm 4.8	137.5 \pm 13.9	N.S.
Mean arterial blood pressure (mmHg)	5	135.1 \pm 11.2	136.5 \pm 1.5	N.S.
Mean peripheral resistance (dynes sec/cm ⁵)		11.56 \pm 10 ³	4.18 \pm 10 ³	
Carotid peripheral resistance (dynes sec/cm ⁵)		59 \pm 10 ⁴	85 \pm 10 ⁴	
Sciatic peripheral resistance (dynes sec/cm ⁵)				

Calculated from mean values of blood flow and arterial blood pressure

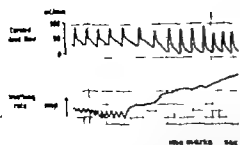


Fig. 2. Palatine carotid blood flow measured at high ambient temperature (35°C). The lower tracing shows the respiratory movements measured with mercury straws. It is evident that when panting suddenly stops, the carotid blood flow increases.

As in the bottom tracings indicating an increase from 20 breaths per minute to 73 breaths per minute associated with panting. The conspicuous increase in carotid blood flow represents an elevation of mean flow from 9.8 ml min^{-1} to 45.1 ml min^{-1} or a 4.6 fold increase. The magnitude of the carotid blood flow increase was variable between specimens (Table 1) as was the rate of the change in blood flow. In some experiments blood flow rose gradually. In other experiments blood flow rose abruptly. In some experiments the major blood flow increase coincided with onset of panting. Upon termination of panting carotid blood flow typically declined abruptly (Fig. 2). Sciatic blood flow did not increase with panting (Fig. 1 Table 1).

Both carotid and sciatic blood flow were very labile. Thus a sound stimulus like opening a door or

tapping on the experimental box would cause a transient increase in both carotid and sciatic blood flow at non-panting condition (left side of Fig. 3). The lack of a sciatic blood flow increase with panting (Fig. 1) further supports the evidence that the increase of carotid blood flow with panting is a truly specific response. When a disturbing sound stimulus was applied during panting, the sciatic blood flow changed in a similar fashion as during non-panting. The greatly increased level of carotid blood flow during panting however abruptly but transiently dropped off when the sound stimulus was applied (Fig. 3 right). Following the stimulus the two flow patterns and breathing rate returned gradually to pre-disturbance values.

Table 1 summarizes results obtained in 9 experiments on different specimens. Sciatic blood flow and systemic arterial pressure were measured in 5 of these experiments.

The rise in body temperature (cloacal temperature) from resting to panting condition usually occurred gradually from the time when the ambient temperature was set to 35°C . The rate of increase in cloacal temperature declined markedly when panting started and in most cases stabilized or even declined during the period of panting.

Table 1 shows that the changes in breathing rate, carotid blood flow and carotid vascular resistance were all highly significant while changes in sciatic blood flow and resistance, systemic arterial pressure and heart rate were not statistically significant. The increased carotid blood flow during panting

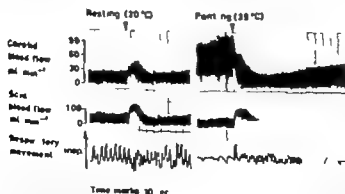


Fig. 3. Recordings of unilateral carotid and sciatic blood flow measured at two different conditions (ambient temperature 20°C) and after 1 hour continued panting at ambient temperature of 35°C . The arrows at the top mark the time when the duck was disturbed by an acoustic stimulus.

ing was always associated with an increased beak temperature

DISCUSSION

Various authors have reported on heart rate and arterial blood pressure associated with thermal panting in birds (Frankel et al 1967 Linsley & Burger 1964 Whitton et al 1964 Harrison & Biellier 1969 Smith 1977 Marder 1973 Bech & Johansen 1980) but this information is not well suited for evaluation of thermoregulatory adjustments in cardiovascular function.

Wolfenson et al (1978a, b) have reported on blood flow distribution in normothermic and hyperthermic hens using radioactive microspheres. Their results showed panting to be associated with marked increases ($\times 15$) in blood flow to the tongue, larynx and trachea.

Many studies of mammals have addressed problems related to changes in regional blood flow at hyperthermic conditions (Ederstrom 1954 Hales 1973 1974 Hales & Dampney 1975 Hales 1976). One series of studies on blood flow changes to the tongue of the dog has particular relevance to the present study (Krönert & Pleschka 1976 Pleschka & Krönert 1976). These studies demonstrated an increase of 3–6 times in lingual blood flow during panting and that this blood flow increase correlated with a greatly increased heat loss from the tongue (Pleschka & Krönert 1976).

The thermoregulatory significance of the directly measured increase in carotid flow presently recorded in panting ducks cannot yet be evaluated nor is it known how much of the blood flow to the head is distributed to the upper respiratory tract during panting. Based on measurements of respiratory water loss and cloacal and surface temperatures Menum & Richards (1975) concluded that the nasal, buccal and upper tracheal surfaces in the fowl were the most important sites of evaporative heat dissipation.

Cardiac output in resting ducks at thermoneutral conditions has been reported by several authors (Sturkie 1966 Folkow et al 1967 Jones & Holeton 1977a, b Scheid et al 1978). Taking a mean value for these studies of 319 ml/min/kg as a reference value for cardiac output in our study, carotid blood flow at thermoneutrality will represent 2.3% of cardiac output. The figure for sciatic flow will be 10.2%. The relatively large fraction of cardiac out-

put directed through the sciatic arteries in birds has been related to the important heat dissipating role of the naked feet (Jones & Johansen 1972). The only published value for cardiac outputs at thermoneutral and panting conditions in a bird appears to be a 6% increase with panting reported for the mute swan (*Cygnus olor*) (Bech & Johansen 1980). We have stipulated cardiac output to increase by 10% in the duck during panting. The fractional distribution of the cardiac output to the carotid and sciatic arteries will then be altered from 2.3% to 3.1% (carotid) and from 10.2% to 8.5% (sciatic) for the resting and panting conditions respectively. Mean systemic arterial pressure remained stable with onset of panting implying an unaltered driving force through the two vascular beds compared. The peripheral vascular resistance must hence have changed in accordance with the altered flow rates decreasing from 11.56×10^5 to 4.18×10^5 dynes sec cm^{-4} for the carotid while the sciatic resistance increased insignificantly from 2.59×10^5 to 2.85×10^5 dynes sec cm^{-4} (Table 1). The non-panting values for vascular resistance compare favourably with values reported by Butler & Jones (1971) on the duck.

An opposite change of vascular resistances between vascular beds serving different functions is commonplace also when one of the vascular beds serves as an effector mechanism in thermoregulation. Thus skin vascular resistance declines while intestinal vascular resistance increases in mammals during heat stress (Schönung et al 1971 Simon 1971). We are unaware however that two vascular beds both important in thermoregulation, such as the sciatic and carotid beds in ducks, have been demonstrated to show a decrease in vascular resistance (the carotid) and an unchanged resistance (the sciatic) during ambient thermal stress.

Baudinette et al (1976) working on the herring gull documented that 37–56% of total heat production at rest was lost via the feet while during flight when metabolic heat production was estimated to be increased 7 times approximately 80% of the total heat production was dissipated across the extremities. During flight blood flow increased about 4 times through the sciatic artery. When comparing these data to ours on the panting duck, one must remember that panting is associated with only a slightly higher heat production (Bouverot et al 1974). Moreover, heat dissipation by convection and conduction becomes less important due to the

lared surface to ambient temperature gradients is the principal problem for the panting bird at rest is not to dissipate more heat, but to alter the modes of heat dissipation from conduction, convection and radiation, to that of evaporation. This change of strategy requires an altered blood flow and thus convection favouring the evaporative surfaces, whereas nothing is gained by an increased perfusion to sites principally involved in heat dissipation by convection and conduction.

REFERENCES

- ALDINETTE, R. V., LOVERIDGE, J. P., WILSON, K. J., MILLS, C. D. & SCHMIDT-NIELSEN, K. 1976 Heat loss from the feet of Herring Gulls at rest and during flight. *Am J Physiol* 230: 970-974.
- BECH, C. & JOHANSEN, K. 1980. Ventilatory and circulatory responses to hyperthermia in the muskrat (*Fiber zibeticus*). *J Exp Biol* (in press).
- OVERDIT, H., HILDWEIN, G. & LEGOFF, D. 1974. Expiratory water loss, respiratory pattern, gas exchange and acid-base balance during thermal panting in Pekin ducks exposed to moderate heat. *Respir Physiol* 21: 251-269.
- ATUL, P. J. & JONES, D. R. 1971. The effect of variation in heart rate and regional distribution of blood flow on the ventral pressure response to diving in ducks. *J Physiol* 214: 457-479.
- EDERSTRÖM, H. E. 1954. Blood flow changes in the leg during hyperthermia. *Am J Physiol* 176: 347-351.
- ROLEWICZ, N., NIELSEN, N. J. & YONCE, L. R. 1967. Effects of diving on cardiac output in ducks. *Acta Physiol Scand* 70: 347-361.
- ROSE, K. E., HOLLANDS, K. G. & WEISS, H. S. 1962. Respiratory and circulatory responses of hyperthermic chickens. *Arch Int Physiol Biochem* 70: 555-563.
- TALES, J. R. S. 1973. Effects of exposure to hot environments on the regional distribution of blood flow and on cardiovascular function in sheep. *Pflügers Arch* 344: 113-148.
- TALES, J. R. S. 1974. Physiological responses to heat. In: *MTP International Reviews of Science Physiology* vol. 7, pp. 107-162. Butterworths, London.
- TALES, J. R. S. 1976. The redistribution of cardiac output in mammals during heat stress. In: *Progress in biometeorology* Ser. B. Annual biometeorology vol. 1 part 1 (ed. H. D. Johanna), pp. 285-294. Swets and Zeitlinger, Amsterdam.
- TALES, J. R. S. & DAMPNEY, R. A. L. 1975. The redistribution of cardiac output in the dog during heat stress. *J Therm Biol* 1: 29-34.
- BRADSHAW, D. C. & BIELLIER, H. V. 1969. Physiological response of domestic fowl to abrupt changes of ambient temperature. *Poultry Sci* 48: 1034-1043.
- JONES, D. R. & HOLETON, G. F. 1972. Cardiovascular and respiratory responses of duck to progressive hypocapnic hypoxia. *J E. Biol* 96: 657-666.
- JONES, D. R. & HOLETON, G. F. 1972b. Cardiac output of duck during diving. *Comp Biochem Physiol* 41A: 639-645.
- JONES, D. R. & JOHANSEN, K. 1972. The blood vascular system of birds. In: *Avian biology* vol. 1 (ed. D. S. Farner and J. R. King), pp. 157-285. Academic Press, New York.
- KRÖNERT, H. & PLESCHKA, K. 1976. Lingual blood flow and hypothalamic control in the dog during panting. *Pflügers Arch* 367: 5-31.
- LASIEWSKI, R. C. 1972. Respiratory function in birds. In: *Avian biology* vol. 1 (ed. D. S. Farner and J. R. King), pp. 287-342. Academic Press, New York.
- LINSLEY, J. G. & BURGER, R. E. 1964. Respiratory and cardiovascular responses in the hyperthermic domestic cock. *Poultry Sci* 43: 791-805.
- MARDER, J. 1973. Temperature regulation in the Bedouin fowl (*Gallus domesticus*). *Physiol Zool* 46: 208-217.
- MENJAN, M. & RICHARDS, S. A. 1975. Observations on the sites of respiratory evaporation in the fowl during thermal panting. *Respir Physiol* 25: 39-47.
- PLESCHKA, K. & KRÖNERT, H. 1976. Thermoregulatory adjustment of lingual blood flow in the conscious dog at high ambient temperatures. *Int J Med Sci* 12: 1077-1078.
- RICHARDS, S. A. 1970. The biology and comparative physiology of thermal panting. *Biol Rev* 45: 223-264.
- SCHMIDT, P., BURGER, R. E., MEYER, M. & GRAF, W. 1978. Diffusion in avian pulmonary gas exchange: role of the diffusion resistance of the blood-gas barrier and the air capillaries. In: *Respiratory function in birds, adults and embryonic* (ed. J. Papez), pp. 136-141. Springer Verlag, Berlin.
- SCHÖNUNG, W., WAGNER, H., JESSEN, C. & SIMON, E. 1971. Differentiation of cutaneous and intestinal blood flow during hypothalamic heating and cooling in anesthetized dogs. *Pflügers Arch* 378: 145-154.
- SIMON, E. 1971. Regional differentiation of vasomotor activity underlying thermoregulatory adjustments of blood flow. *Int J Biometeor* 15: 219-224.
- SMITH, R. M. 1972. Circulation, respiratory volumes and temperature regulation of the pigeon in dry and humid heat. *Comp Biochem Physiol* 43 A: 477-490.
- STURKIE, P. D. 1966. Cardiac output in ducks. *Proc Soc Exp Biol Med* 123: 487-488.
- WHITTON, G. C., STURKIE, P. D. & STEIN, D. 1964. Cardiovascular changes associated with thermal polymorphism in the chicken. *Am J Physiol* 207: 1349-1353.
- WOLFENSON, D., BERMAN, A., FREI, Y. F. & SNAPE, N. 1978a. Measurement of blood flow distribution by radioactive microspheres in the laying hen (*Gallus domesticus*). *Comp Biochem Physiol* 61A: 549-554.
- WOLFENSON, D., BERMAN, A., FREI, Y. F. & SNAPE, N. 1978b. Heat-stress effects on regional blood flow distribution in the laying hen, measured by radioactive microspheres. *Int J Med Sci* 14: 1091.

ing was always associated with an increased beak temperature.

DISCUSSION

Various authors have reported on heart rate and arterial blood pressure associated with thermal panting in birds (Frankel et al. 1962, Linsley & Burger 1964, Whittow et al. 1964, Harrison & Biellier 1969, Smith 1977, Marder 1973, Bech & Johansen 1980) but this information is not well suited for evaluation of thermoregulatory adjustments in cardiovascular function.

Wolfenson et al. (1978a, b) have reported on blood flow distribution in normothermic and hyperthermic hens using radioactive microspheres. Their results showed panting to be associated with marked increases ($\times 15$) in blood flow to the tongue, larynx and trachea.

Many studies of mammals have addressed problems related to changes in regional blood flow at hyperthermic conditions (Ederstrom 1954, Hales 1973, 1974, Hales & Dampney 1975, Hales 1976). One series of studies on blood flow changes to the tongue of the dog has particular relevance to the present study (Krönert & Pleschka 1976, Pleschka & Krönert 1976). These studies demonstrated an increase of 5–6 times in lingual blood flow during panting and that this blood flow increase correlated with a greatly increased heat loss from the tongue (Pleschka & Krönert 1976).

The thermoregulatory significance of the directly measured increase in carotid flow presently recorded in panting ducks cannot yet be evaluated, nor is it known how much of the blood flow to the head is distributed to the upper respiratory tract during panting. Based on measurements of respiratory water loss and cloacal and surface temperatures, Mennam & Richards (1975) concluded that the nasal, buccal and upper tracheal surfaces in the fowl were the most important sites of evaporative heat dissipation.

Cardiac output in resting ducks at thermoneutral conditions has been reported by several authors (Sturkie 1966, Folkow et al. 1967, Jones & Hopton 1972a, b, Scheid et al. 1978). Taking a mean value for these studies of 379 ml min⁻¹ kg⁻¹ as a reference value for cardiac output in our study, carotid blood flow at thermoneutrality will represent 2.3% of cardiac output. The figure for sciatic flow will be 10.2%. The relatively large fraction of cardiac out-

put directed through the sciatic arteries in birds has been related to the important heat dissipating role of the naked feet (Jones & Johansen 1972). The only published value for cardiac outputs at thermoneutral and panting conditions in a bird appears to be a 11% increase with panting reported for the mute swan (*Cygnus olor*) (Bech & Johansen 1980). We have stipulated cardiac output to increase by 10% in the duck during panting. The fractional distribution of the cardiac output to the carotid and sciatic arteries will then be altered from 2.3% to 5.8% (carotid) and from 10.2% to 8.5% (sciatic) for the resting and panting conditions, respectively. Mean systemic arterial pressure remained stable with onset of panting implying an unaltered driving force through the two vascular beds compared. The peripheral vascular resistance must hence have changed in accordance with the altered flow rates, decreasing from 11.56×10^6 to 4.18×10^6 dynes sec cm⁻⁵ for the carotid, while the sciatic resistance increased insignificantly from 2.59×10^6 to 2.85×10^6 dynes sec cm⁻⁵ (Table 1). The non-panting values for vascular resistance compare favourably with values reported by Butler & Jones (1971) on the duck.

An opposite change of vascular resistances between vascular beds serving different functions is commonplace also when one of the vascular beds serves as an effector mechanism in thermoregulation. Thus skin vascular resistance declines while intestinal vascular resistance increases in mammals during heat stress (Schönung et al. 1971, Simon 1971). We are unaware, however, that two vascular beds both important in thermoregulation, such as the sciatic and carotid beds in ducks, have been demonstrated to show a decrease in vascular resistance (the carotid) and an unchanged resistance (the sciatic) during ambient thermal stress.

Baudinette et al. (1976) working on the herring gull documented that 37–56% of total heat production at rest was lost via the feet, while during flight when metabolic heat production was estimated to be increased 7 times, approximately 80% of the total heat production was dissipated across the extremities. During flight blood flow increased about 4 times through the sciatic artery. When comparing these data to ours on the panting duck, one must remember that panting is associated with only a slightly higher heat production (Bouverot et al. 1974). Moreover, heat dissipation by convection and conduction becomes less important due to the

Relation between cell length and force production in urinary bladder smooth muscle

HENGT UVELIUS and GIORGIO GABELLA

Department of Physiology University of Lund, Sweden, and Department of Anatomy University College London, Great Britain

UVELIUS, H. & GABELLA, G. Relation between cell length and force production in urinary bladder smooth muscle. *Acta Physiol Scand* 1980 110 357-365. Received 18 Feb 1980, ISSN 0001-6772. Department of Physiology University of Lund, Sweden, and Department of Anatomy University College London, Great Britain.

Guinea-pig and rabbit urinary bladders were fixed in glutaraldehyde at different volumes. Strips were dissected out, embedded and cut for plane contrast and electron microscopy. Muscle wall thickness decreased with increased bladder volume as did the radial number of muscle cells. Cell length, measured by a morphometric method increased linearly with bladder radius, indicating that no slippage between the muscle cell occurred. Number of cells per μm^2 cross-sectional area increased linearly with bladder radius. Volume-active force relations were obtained by pelvic nerve stimulations of guinea pig bladders filled to different volumes. Maximum pressure was obtained at 0.15 ml bladder volume and maximum wall tension at volume of 2.5 ml which corresponds to cell length of 400 μm and cell packing density of about 107 000 mm^{-2} . Extension of the length-active tension curve for the average muscle cell in the guinea-pig bladder indicated a maximum active force of 5.5 $\mu\text{N}/\text{cell}$. Maximum active force per cm^2 muscle bundle was calculated to be about 99 N. No compensation for intracellular space and nonmuscular tissue within the muscle bundle was made.

Key words: Urinary bladder, cell length, length-tension relations, cell force.

In vertebrate smooth muscles the maximum force developed per unit sectional area can equal or even exceed that of a skeletal muscle (Murphy 1976, Mulvany & Halpern 1976, Gabella 1976b). In spite of their relatively low myosin content (Murphy, Herlihy & Meyerson 1974) the number of studies in this field is small. The taenia coli of the guinea-pig and some arterial arteries are the tissues most commonly investigated. It would clearly be interesting to assess the force development in other smooth muscles, particularly in those in which there is a different geometrical arrangement of muscle cell than in taenia coli or arterial media.

Smooth muscles consist of cells that are coupled in each other in a complex manner (see Gabella 1977). In order to analyze the results of experiments on muscle strips in terms of behaviour of the individual cells it is necessary to know how the length of the cells varies with the length of the preparation. In earlier studies (Cooke & Fay 1972, Uvelius 1976, Džurka & Murphy 1978) smooth muscles stretched to different lengths were macerated with nitric acid

or KOH and the length of individual isolated muscle cells were measured. These studies suggest that the cells are essentially coupled in series, that is the changes in cell length mirror the changes in length of the muscle strip. A limitation of the method used is that the length of the cells may be affected by the maceration. Halpern, Mulvany & Warsaw (1978) measured with interference microscopy stretch-induced changes in the distance between intracellular markers in muscle cells of small mesenteric arteries in vitro. They found a linear relationship between the changes in the separation of the intracellular markers and the circumference of the vessel. It remains to be established however whether the same holds true for the tapering parts of the cell.

With the present experiments we aimed to measure the maximum force generated by the detrusor muscle of the bladder (a muscle which can be set up in vivo without direct surgical interference with the tissue). In addition, by applying morphometric techniques in light and electron microscopy we wanted to calculate the force generated

Relation between cell length and force production in urinary bladder smooth muscle

BENGT UVELIUS and GIORGIO GABELLA

Department of Physiology, University of Lund, S-223 62, and Department of Anatomy, University College London, Great Britain

UVELIUS B & GABELLA G. Relation between cell length and force production in urinary bladder smooth muscle. *Acta Physiol Scand* 1980; 110: 357-365. Received 11 Feb. 1980. ISSN 0001-6772. Department of Physiology, University of Lund, Sweden, and Department of Anatomy, University College London, Great Britain.

Guinea-pig and rabbit urinary bladders were fixed in glutaraldehyde at different volumes. Strips were dissected out, embedded and cut for phase contrast and electron microscopy. Muscle wall thickness decreased with increased bladder volume as did the radial number of muscle cells. Cell length, measured by a morphometric method increased linearly with bladder radius, indicating that no slippage between the muscle cells occurred. Number of cells per mm² cross sectional area increased linearly with bladder radius. Volume-active force relations were obtained by pelvic nerve stimulation of guinea pig bladders filled to different volumes. Maximum pressure was obtained at 0.15 ml bladder volume and maximum wall tension at volume of 2.5 ml which corresponds to a cell length of 400 μ m and cell packing density of about 107 000/mm². Estimation of the length-active tension curve for the average muscle cell in the guinea-pig bladder indicated maximum active force of 5.5 μ N/cell. Maximum active force per cm² muscle bundle was calculated to be about 39 N. No compensation for extracellular space and nonmuscular tissue within the muscle bundle was made.

Key words: Urinary bladder, cell length, length-tension relations, cell force.

In vertebrate smooth muscles the maximum force developed per unit sectional area can equal or even exceed that of a skeletal muscle (Murphy 1976; Mulvany & Halpern 1976; Gabella 1976b). In spite of their relatively low myosin content (Murphy, Herlihy & Meyerman 1974). The number of studies in this field is small. The tissues of the guinea-pig and some arterial muscles are the tissues most commonly investigated. It would clearly be interesting to assess the force development in other smooth muscles, particularly in those in which there is a different geometrical arrangement of muscle cells than in taenia coli or arterial media.

Smooth muscles consist of cells that are coupled to each other in a complex manner (see Gabella 1977). In order to analyze the results of experiments on muscle strips in terms of behaviour of the individual cells it is necessary to know how the length of the cells varies with the length of the preparation. In earlier studies (Cooke & Pay 1972; Uvelius 1976; Droks & Murphy 1978) smooth muscles stretched to different lengths were macerated with nitric acid

or KOH and the length of individual isolated muscle cells were measured. These studies suggest that the cells are essentially coupled in series, that is the changes in cell length mirror the changes in length of the muscle strip. A limitation of the method used is that the length of the cells may be affected by the maceration (Halpern, Mulvany & Warshaw (1978) measured with interference microscopy stretch-induced changes in the distance between intracellular markers in muscle cells of small mesenteric arteries in vitro. They found a linear relationship between the changes in the separation of the intracellular markers and the circumference of the vessel. It remains to be established, however, whether the same holds true for the tapering parts of the cell).

With the present experiments we aimed to measure the maximum force generated by the detrusor muscle of the bladder (a muscle which can be set up in vivo without direct surgical interference with the tissue). In addition, by applying morphometric techniques in light and electron microscopy we wanted to calculate the force generated

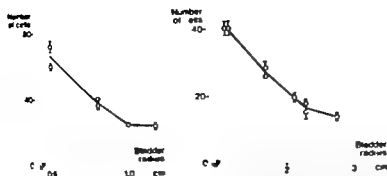


Fig. 1. Number of muscle cells across the bladder wall vs. outer radius for guinea-pig (left) and rabbit (right) bladders. Mean \pm S.E. (25 determinations for each bladder). 6 guinea-pig bladders and 8 rabbit bladders were used. The number of cells across the wall decreases as the bladder volume is increased except for the highest volumes.

bladder wall lies along with the circular musculature in the center of the field in the bladder assuming spherical symmetry. All data are expressed as mean \pm standard error (S.E.).

RESULTS

Thickness of the muscle coat and number of cells

The thickness of the wall of the bladder and that of its muscle coat decreased with the distension of the bladder. With an increase in the bladder volume and corresponding distension of the wall the bundles of muscle cells spread tangentially; the transverse sectional profiles of the bundles tended to be round in empty bladders, whereas they tended to flatten in distended bladders (Plate 1). As a consequence of this re-arrangement the number of muscle cells across the thickness of the wall (see Methods) decreased when the organ is inflated (Fig. 1). In other words, with distension of the organ, the muscle coat becomes thinner and, at any one point, is formed by a smaller number of cells. With distension of the bladder the profiles of individual muscle cells (in transverse section) became smaller in area. This change is borne out by the electron micrographs (Plate 1) and was expressed in a quantitative way by counting the number of muscle cell profiles per unit sectional area of muscle coat (cell packing density). Muscle bundles which were in exact transverse section (as confirmed by cutting a series of sections) were used. In the guinea-pig the packing density ranged from 36 000 cells per mm^2 in nearly empty bladders to 170 000 cells per mm^2 in distended bladders (Fig. 2). In the rabbit at 5 ml bladder volume

the packing density in two experiments was 86 000 and 64 000 per mm^2 respectively. At 20 ml 117 000 and 141 000 cells per mm^2 were counted in two experiments.

Lengths of smooth muscle cells

The approximate length of the muscle cells was estimated with a technique (see Methods) based on measurements on longitudinal and transverse sections from adjacent areas of the same tissue block. The results show a correlation between radius of the bladder and muscle cell length (Fig. 3). In both guinea-pig and rabbit the range is 160 to 580 microns (corresponding to radii of 0.4 cm and 1.2 cm).

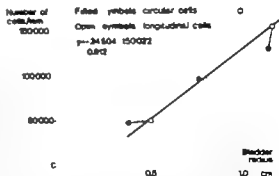


Fig. 2. Packing density of muscle cells. Guinea-pig. Number of cross sectioned longitudinal and circular muscle cells per mm^2 vs. bladder inner (circular muscle) and outer radius (longitudinal muscle). Filled symbols indicate circular muscle and open symbols longitudinal. Symbols connected by broken lines indicate determinations from the same bladder. The best fitting straight line by linear regression analysis for the entire material is shown. 6 bladders were used. Each point is based on 184–405 cells.

by the individual intact muscle cells *in situ*. Direct measurements of force in single muscle cells have been made by Fay (1977) on muscle cells enzymatically isolated from the toad stomach.

METHODS

Material

Adult guinea-pigs (body weight 300–500 g) and rabbits (body weight 2 500–3 500 g) were used.

Histology

For the structural studies the animal was stunned and bled. The abdomen was opened longitudinally from pubis to sternum and the urinary bladder exposed. In some experiments the urethra was ligated to prevent emptying of the urine inside the bladder; the bladder was then dissected out, immersed for 10 min in oxygenated Krebs solution at about 30°C and fixed. The volume of the lumen was measured as the volume of urine collected when the bladder was slit open after about 15 min in fixative. The above procedure gave poor preservation of the fine structure of muscle cells and was therefore modified by replacing the urine with Krebs solution through a cannula inserted into the urethra before excision of the bladder. The emptying of the bladder was carried out in small steps and the refilling was carried out through a gravity fall not greater than 10 cm of water. The highest degrees of bladder filling required up to 30 min to become complete. The bladder was then fixed by transferring it from Krebs solution to the fixative solution. The latter was at room temperature and consisted of 5% glutaraldehyde in 100 mM Na cacodylate at pH 7.4 with 0.4 mM CaCl_2 . After about 10 min in fixative the bladder was slit open, cut into identifiable portions (8 to 14 portions for each bladder) and transferred into fresh fixative. The fixation procedure lasted ~6 h after which the specimens were washed for 15 min in cacodylate buffer and immersed for 60–90 min in 2% osmium tetroxide in cacodylate buffer. The specimen were then block stained in a 1% aqueous solution of uranyl acetate for 30 min at room temperature and dehydrated in graded ethanol and epoxypropene and infiltrated in Araldite. Sections for light microscopy (1.5–3 μm thick) were cut with glass knives, collected on microscope slides, examined and photographed unstained in a phase contrast microscope. Section for electron microscopy cut with glass knives were collected on copper grid stained with lead citrate and examined in a Philips 300 electron microscope. All the histological observations were carried out on a region of the bladder equidistant from the upper and the lower poles (equatorial region) and particularly from the ventral part.

Morphometry

Measurements of the thickness of the muscle coat were carried out on photographic prints or directly in the microscope with the aid of a calibrated eye-piece. The numbers of muscle cells present across the full thickness of the muscle coat were counted by counting the numbers of muscle cells intersected by radial lines drawn orthogonally through the wall. The transverse sectional area of the

muscle bundles was measured with the aid of a planimeter on photographic prints and the number of nuclei per profiles within a bundle counted with the aid of a cube counter. The average length of smooth muscle cells estimated with the method described in detail elsewhere (Gabella 1976a). The method is based on counting percentage of nucleated cell profiles in low powered micrographs of a transversely sectioned bundle, measuring the length of nuclei in longitudinal sections, an adjacent portion of the same muscle bundle examined in light microscopy. The number of cells counted for determination ranged between 448 and 257 (mean 138) and the number of nuclei between 76 and 115. We have made corrections for the shrinkage of the tissue and for compression of the sections. In smooth muscles these factors introduce only a small error in the assessment of area and lengths, and do not affect our results which are comparisons between uniformly processed tissues.

Pressure-volume experiments

The experiments were carried out on ether-anesthetized guinea pigs weighing about 400 g. A mid-ventral section 1 cm long exposed the bladder which was then cannulated through the urethra. The bladder was emptied and held leaving the cannula in place. After ligation the ureters were cut off at 0.5–1 cm distance from the bladder as were drawn together with adjoining tissue including the pelvic nerve through a 0.5 cm long plastic tube with two platinum electrodes at each end. The electrodes from outside were connected to a Grass S44 stimulator via a Grass SIU5 stimulus isolation unit. The cannula was connected to a Statham P23D₁ pressure transducer placed at the same level as the bladder. Through a branch tube the bladder was filled with Krebs solution by gravity to desired volumes. After the pressure had stabilized the nerve supply of the bladder was supramaximally stimulated on both sides by a train of square waves (usually 30 V, 2 ms duration, 30 Hz) for 1 s. This period was long enough to allow the bladder to produce a pressure plateau. The bladder was then filled to a different volume and the sequence was repeated. When the pressure for the higher desired volume had been obtained the bladder was emptied in steps and stimulated again at each volume. The time between each stimulation period was 10 min. Repeated stimulations under these conditions, but with constant bladder volume, gave almost identical pressure values for more than one hour.

For sake of simplicity we have treated the urinary bladder as spherical in shape. In the guinea-pig the bladder is practically no-dimensional at all degrees of distension. On the other hand, the bladder of the rabbit becomes, with extensive distension, slightly elongated along an axis from the upper (cranial) to the lower (caudal) pole. When dealing with the longitudinal musculature (which is located closer to the exterior than to the interior of the bladder wall) the radius of the bladder is expressed as bladder outer radius. This is obtained by adding the volume of the bladder tissue to the fluid volume in the bladder and calculating the radius for this total volume. In other experiments bladders were weighed. In guinea-pigs bladders weigh about 0.3 g and thus have a volume of about 0.3 ml. Rabbit bladders have a weight of about 1.8 g. The inner

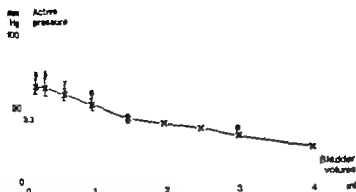


Fig. 4. In vivo experiments of bilateral pelvic nerve stimulation. Active pressure vs. bladder inner volume. Guinea-pig, 4 animals were used. Mean \pm S.E. Number of determinations indicated in the figure. The maximum active pressure is achieved at a bladder volume of 0.15 ml.

section 4) by this plane and from the values of cell length as a function of bladder volume (or radius) (Fig. 3) the length-tension relation of single muscle cells can be calculated (Fig. 6). The maximum active tension per cell is about 5.5 μ N. Using the highest and the lowest estimates of the total number of cells (section 4) the maximum tension per cell ranges between 5.0 and 6.0 μ N.

DISCUSSION

In this study we combined a morphological and a physiological method to study the generation of force in the detrusor muscle of the bladder. With

increase in volume of the bladder the wall is distended and the muscle coat becomes thinner. At the same time the muscle cells become thinner and their length increases. The morphometric study indicates that over most of the wide volume range investigated there is a linear relation between bladder radius and muscle cell length. It is therefore unlikely that there is any significant longitudinal slippage or sliding of the cells past each other when the bladder is distended, a result which is in agreement with previous observations on rabbit urinary bladder (Uvelius 1976) and on other smooth muscles (Cooke & Fay 1977; Driska & Murphy 1978). The relation between cell length and bladder radius de-

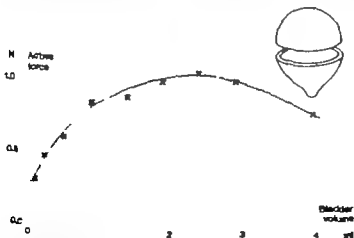


Fig. 5. Force produced by the longitudinal myoelectricity holding the two indicated hemispheres together vs. bladder inner volume. The force developed is maximal at bladder volume of 2.5 ml.

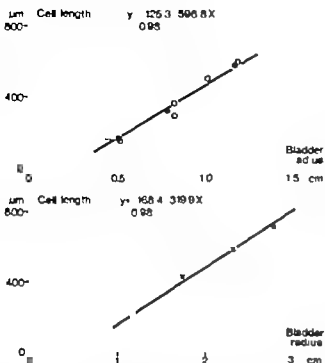


Fig. 3. Estimated cell length of smooth muscle cells vs. inner radius (circular muscle cells) or outer radius (longitudinal muscle cells). Upper diagram, guinea-pig six bladders. Filled symbols indicate circular and open symbols longitudinal muscle. Connected symbols indicate determinations from the same bladder. Lower diagram, Rabbit eight bladders. Longitudinal muscle. The best fitting lines obtained by linear regression analysis are shown.

in the guinea-pig and 700 to 700 (radius of 1.1 cm and 7.8 cm) in the rabbit.

3. Volume-pressure relation

In the guinea-pig the maximum active pressure following pelvic nerve stimulation amounted to about 65 ± 5 mmHg and it was reached at a bladder volume of 0.15 ml. The pressure developed upon stimulation steadily declined with greater distension of the organs, reaching a value of about 30% of maximum when the volume of the bladder was 4 ml (Fig. 4). At all bladder volumes examined the passive pressure was small compared to the active pressure: at a bladder volume of 4 ml it amounted to about 3 mmHg.

4. Force developed by the bladder wall

The relation between pressure in the lumen and force developed by the wall of the organ is expressed by the equation $F = r^2 \times \pi \times P$, where F is the force in Newton, P is pressure in Pascal, and r is the radius of the spherical organ (F being the force trying to separate the two hemispheres). The force

developed inside the wall to antagonize the effect of the luminal pressure obtained during contraction, produced by the bundles of smooth muscle holding the two hemispheres together. This force is expressed by the equation $F_b = A \times T$, where A is the area of cross sectioned muscle and T is the tension produced per unit area of this muscle. If the volume of the bladder is maintained constant as in the present experiments $F = F_b$. From the above equation and by using the pressure values of Fig. 4 the active force developed by the wall of the bladder following nerve stimulation has been calculated as a function of the volume of the bladder (Fig. 4). The maximal force holding the two hemispheres together is 1.04 N and is attained at about 7.8 ml bladder volume.

Some uncertainty in this calculation is introduced by the measurements of the transverse section area of the "longitudinal" musculature slightly obliquely sectioned muscle bundles whose "longitudinal" pull is less effective than that of transversely sectioned bundles were included whereas highly obliquely sectioned bundles (which most likely contribute some force in the "longitudinal" direction) were excluded. With this source of uncertainty in mind we refer to the cells which effectively pull the two hemispheres together being "approximated in transverse section". In order to calculate the force developed by a single muscle cell from the tension produced by the whole bladder (see section 5) an estimate of the total number of cells pulling the two hemispheres together was needed. The total number of muscle cells which were approximated in transverse section in an equatorial plane of the guinea-pig bladder at various volumes (calculated from the cell packing density, the total section area of the muscle coat and the percentage of approximately transversely sectioned bundles) was 185 000 (mean of 8 determinations \pm S.E. = 74 000). The scatter is high and there is a tendency towards an increase in number of cells with increase in bladder volume. We consider this trend as reflecting the uncertainty and inaccuracy of the counting methods.

5. Maximum active tension per cell

If we consider an equatorial plane through the guinea-pig bladder the force generated at various bladder volumes can be calculated from the tension produced by the "longitudinal" muscle pulling the two hemispheres together. From the number of cells approximately transversely sectioned (185 000,



Fig. 2. Electron micrograph of the musculature of guinea pig bladder with content volume of 2.0 ml. A bundle of muscle cells in transverse section (to the left) and one in highly oblique section (to the right). Bar 10 μ m.

transverse sectional area indicate a linear relation between bladder radius and cell packing density (cells/mm²). Distension of the wall is thus accompanied by a proportional increase in length of

the individual muscle cells and the elongated muscle cells have a proportionally thinner transverse sectional profile and therefore an increased packing density. Two other structural changes in the muscle

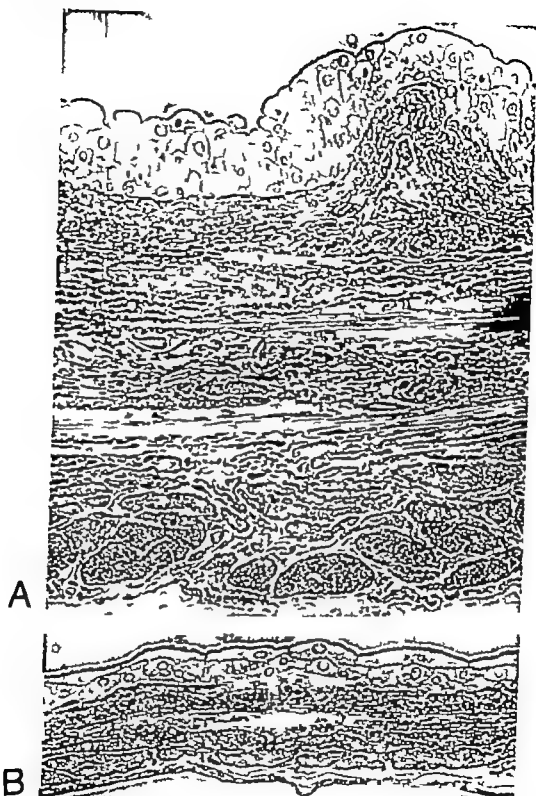


Plate 1 Phase contrast micrographs of the wall of the rabbit urinary bladder (full thickness). In A the content volume was 5 ml, in B it was 40 ml. The epithelium is at the top in both micrographs. Bar 100 μ m.

varies from this linearity at very small bladder volumes, but here the thickness of the bladder wall is very large compared with the volume of the lumen and probably a more complex structural model

should be used (one probably involving different activity in concentric layers of musculature). Estimates of cell volume were not carried out, but the measurements of packing density of muscle cells

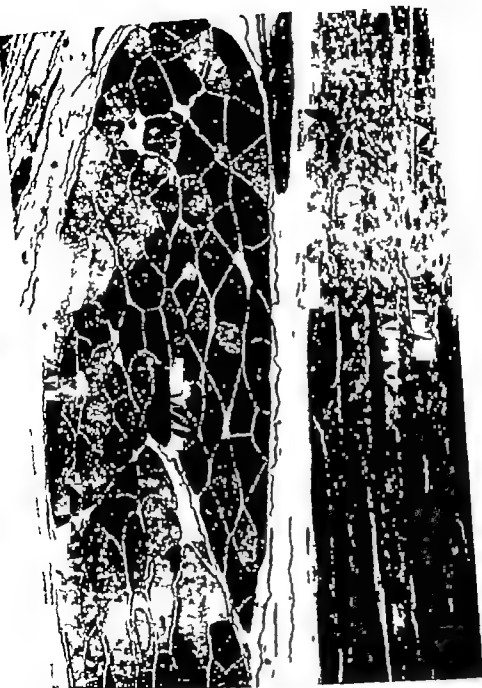


Fig. 2. Electron micrograph of the musculature of guinea pig bladder with a content volume of 2.0 ml. A bundle of muscle cells in transverse section (to the left) and one in highly oblique section (to the right). Bar 10 μ m.

per unit transverse sectional area indicate a linear relation between bladder radius and cell packing density (cells/ mm^2). Distension of the wall is thus accompanied by a proportional increase in length of

the individual muscle cells and the elongated muscle cells have a proportionally thinner transverse sectional profile and therefore an increased packing density. Two other structural changes in the muscle

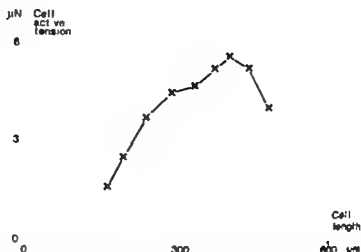


Fig. 6. Calculated length-tension relation for the average individual longitudinal muscle cell.

coat accompany the distension of the wall. The transverse sectional profiles of the bundles of muscle cells tend to be round at small bladder volumes but are flattened tangentially at large bladder volumes (Plate 1) and so are the profiles of the individual muscle cells. Moreover with distension of the wall there is some spatial re-organization of the bundles of muscle cells—a set of changes that we do not yet understand well but which involve some lateral sliding of muscle bundles over each other.

The pressure-volume relations obtained are qualitatively similar to those obtained by others (see e.g. Carpenter 1968; de Sy 1971). The estimates of force produced by the individual muscle cell were derived from these measurements of bladder pressure and from the histological data but they required a number of assumptions to be made. The assumption that the bladder can be considered a sphere is reasonable for the guinea-pig (whereas in the rabbit the bladder is somewhat elongated from pole to pole) and the distensibility of the wall appears to be uniform throughout the organ with the exception of the relatively small region of the trigonum which is less stretchable. We also assumed that—except at very small bladder volumes—all the muscle cells of the organ behaved mechanically uniformly, namely that they were all of the same length and contracted to the same degree. The estimate of the total number of muscle cells developing tension across the equatorial plane was somewhat uncertain because of the presence of obliquely arranged bundles of cells. Moreover the estimates of maximum force refer strictly to the experimental conditions used (bilateral electrical

nerve stimulation) and do not imply that this is the maximum absolute force that the tissue is capable of producing. It is possible that under other conditions more powerful contractions may take place. The very concept of maximum force remains difficult to define for smooth muscle since it depends on the type of stimulation and the excitability and the metabolic conditions of the tissue and in a more general way on the past history of the muscle. An advantage of our experimental approach was that the force measurements could be carried out without direct interference with the organ. The vascular supply was intact and no cuts had to be made in the muscle.

Maximum active force for guinea-pig bladder was produced at a volume of 7.5 ml (Fig. 5) corresponding to an outer radius of 0.88 cm. This corresponds according to Fig. 3A to a cell length of about 400 μm. At this length the estimates of cell force range between 5 and 6 μN. Other studies of force per cell have reported values of 5 μN in vascular smooth muscle (Mulvany & Halpern 1976) and 2.3 μN in rabbit urinary bladder (Uvelius 1976). The latter estimate is lower than the one we have obtained in the present experiments on guinea-pig bladder. We think that the difference may be due to some damage inevitably produced in the dissection and mounting of the rabbit muscle strips in the earlier experiments (Uvelius 1976). On this account the present experiments are more reliable since they involve no direct interference with the organ during the measurements of force. The maximum force produced per cell is comparable to those obtained by Fay (1979) on isolated muscle cells from toad

smooth is his study values from 2 μN and upwards as obtained giving a mean value of 2.6 N/ cm^2 when correction as made for cross sectional area.

The number of cells per mm^2 amounts to 107 000 (radius of ± 0.075 cm (Fig. 2). The active force per cm^2 of cross sectional area would then be about 59 l. This value is higher than that in isolated strips of the urinary bladder (19 N $\times\text{cm}^{-2}$) (Uellius 1976) mainly on account of the more appropriate way of making the muscle adopted in the present experiments. The present value of 59 N $\times\text{cm}^{-2}$ is fairly close to those obtained for the media of the pig aorta artery (37 N $\times\text{cm}^{-2}$) (Murphy et al. 1974) and for the guinea-pig taenia coli (4 N $\times\text{cm}^{-2}$) (Gabella 1976b). The latter value is increased to about 74 N $\times\text{cm}^{-2}$ when the force is expressed per unit area of contractile material. When allowance is made for the different experimental approaches and for some uncertainty in the notion of maximum force, it appears that the maximum amounts of force measured vary rather little in the different tissues tested. In these arterial, intestinal and urinary muscles the values are as high or higher as those calculated for striated muscle fibres (Close 1972).

This work was supported by grants from the British Medical Research Council, the Swedish Medical Research Council and from the Medical Faculty, University of Lund, Sweden.

REFERENCES

- CARPENTER, F. G. 1968 Motor responses of bladder smooth muscle as relation to elasticity and fiber length. *J. Invest. Biol.* 6, 273-283.
- CLOSE, R. J. 1972. Dynamic properties of mammalian skeletal muscles. *Physiol. Rev.* 52, 129-197.
- COOKE, P. H. & FAY, F. S. 1972. Correlation between fiber length, ultrastructure and the length-tension relationship of mammalian smooth muscle. *J. Cell Biol.* 12, 105-116.
- DRISKA, S. P. & MURPHY, R. A. 1978. Estimate of cellular force generation in an arterial smooth muscle with high actin: myosin ratio. *Blood Vessels* 15, 26-32.
- FAY, F. S. 1977. Isometric contractile properties of single isolated smooth muscle cells. *Nature* 265, 553-556.
- GABELLA, G. 1976a. Quantitative morphological study of smooth muscle cells of the guinea-pig taenia coli. *Cell Tiss. Res.* 170, 161-186.
- GABELLA, G. 1976b. The force generated by visceral smooth muscle. *J. Physiol.* 263, 199-213.
- GABELLA, G. 1977. Arrangement of smooth muscle cells and intramural septa in the taenia coli. *Cell Tiss. Res.* 184, 195-212.
- HALPERN, W., MULVANY, M. J. & WARSHAW, D. M. 1978. Mechanical properties of smooth muscle cells in the walls of arterial resistance vessels. *J. Physiol.* 275, 85-101.
- MULVANY, M. J. & HALPERN, W. 1976. Mechanical properties of vascular smooth muscle cells in situ. *Nature* 260, 617-619.
- MURPHY, R. A. 1976. Contraction system function in mammalian smooth muscle. *Blood Vessels* 13, 1-23.
- MURPHY, R. A., HERLIHY, J. T. & MIEGERMAN, J. 1974. Force-generating capacity and contractile protein content of arterial smooth muscle. *J. Gen. Physiol.* 64, 691-705.
- DE SY, W. 1971. The reactivity of isolated urinary bladder strips of the guinea-pig towards electrical stimulation. *Arch. Int. Physiol. Biochem.* 79, 459-468.
- UELLIUS, B. 1976. Isometric and heliocentric length-tension relations and variations in cell length in longitudinal smooth muscle from rabbit urinary bladder. *Acta Physiol. Scand.* 97, 1-12.

Vanadate-induced oliguria and vasoconstriction in the cat

ES ANKER LARSEN and OLE ØSTERGAARD THOMSEN

Institute of Physiology, University of Aarhus, DK-8000 Aarhus C, Denmark

LARSEN J. A. & THOMSEN O. Ø. Vanadate-induced oliguria and vasoconstriction in the cat. *Acta Physiol Scand* 1980, 110: 367-374. Received ... Febr 1980. ISSN 0001-6772. Institute of Physiology, University of Aarhus, Denmark.

The effect of sodium orthovanadate (vanadate) on kidney function and hemodynamics was tested in chloralose anesthetized, fasting cats. Vanadate given caused reversible marked decrease in glomerular filtration rate and urine production without significantly affecting the fractional water excretion rate. In the recovery period, after administration of vanadate, urine flow and the urinary excretion rate of electrolytes were only slightly different from control values. This action of vanadate appears to be due mainly to constriction of the renal blood vessels leading to a fall in glomerular capillary pressure. Vanadate like was caused dose dependent decrease in arterial conductance and blood flow of other vessels in the splanchnic area, whereas the femoral and carotid arteries were reversibly and less affected. The vascular effect appears to be caused by direct action of vanadate on vascular smooth muscle, but the mechanism of action is still unsettled.

Key words. Kidney function, vanadate, glomerular filtration rate, hemodynamics.

In a previously published short communication we reported that vanadate caused a marked decrease in urine production in the cat (Larsen et al. 1979) in contrast to the diuretic effect of vanadate observed in rats (Balkov et al. 1978). The oliguric effect appeared to be due to a direct constrictive action of vanadate on the renal blood vessels leading to a fall in glomerular capillary pressure.

The present publication provides additional evidence that vanadate-induced oliguria is caused predominantly by a fall in glomerular capillary pressure. The results also demonstrate that vanadate is a potent constrictor of other vascular areas in the cat.

METHODS

Young cats weighing approx. 3.0 kg were fasted overnight and anesthetized with chloralose (50 mg/kg) and a minimal dose of Nembutal (30 mg). Body temperature was kept close to 38°C by external heating.

Catheters for infusions and blood sampling were placed in the femoral veins and arteries. Mean arterial blood pressure was recorded from the femoral artery by means of a Statham transducer (Elema Schöander). The abdominal aorta was exposed by midline incision and when kidney

function was followed, catheter was placed either in the urinary bladder or in one or both ureters, and urine was collected in 5-10 min periods. When hepatic artery blood flow was measured, the common hepatic artery was dissected free and the right gastro-duodenal artery ligated. Flow was then measured in the common hepatic artery. In some experiments catheter was placed in the portal vein via peripheral anastomotic vein for blood pressure measurement.

Blood flow was measured by means of an electromagnetic flowmeter (Nycotron Oslo) and the probe was placed on the vessel after removal of surrounding connective tissue and nerves. Zero adjustment was performed according to the manufacturer's manual and calibration made on the femoral artery and abdominal aorta. Pressure and flow were recorded simultaneously on Beckman 5 II Dynograph.

When kidney function was examined 50 ml of 0.6% NaCl was given at the start of the experiment in order to stimulate urine flow. Glomerular filtration rate was estimated from urinary inulin clearance. Inulin was given as priming dose of 800 µg unlabelled inulin and 1 µCi ¹²⁵I-inulin in 8 ml 0.9% NaCl followed by continuous infusion of 10 µg unlabelled inulin and 0.01 µCi ¹²⁵I-inulin in 0.1 ml 0.9% NaCl per min.

Vanadate (sodium orthovanadate) was dissolved in isotonic sodium chloride and given either as a single dose (1 ml in 1 min) or continuously (0.1 ml/min).

When the operative procedures were finished, re-

Table 1. Kidney function before (A), during (B) and after (C) infusion of vanadate $0.5 \mu\text{mol kg}^{-1} \text{ min}^{-1}$. Urine was collected from the right (r) and left (l) ureter in 2 expts. and from only one ureter in 3 experiments

	Urine flow (mg kg ⁻¹ min ⁻¹)			¹⁴ C-inulin urine ¹⁴ C-inulin plasma			¹⁴ C-inulin clearance (mg kg ⁻¹ min ⁻¹)		
	A	B	C	A	B	C	A	B	C
1	28.3	0.9	4.3	111	177	706	1 128	145	876
1	71	0.7	6.1	141	177	170	990	76	836
2	5.9	0.3	4.0	283	226	41	1 663	135	940
1	48	0.0	4.2	45	478	227	1 166	96	924
1	55	0	5.6	309	556	268	1 690	111	1 484
1	43	0.1	10.4	393	313	130	1 679	31	1 308
Mean	63	0.4	5.7	47	31	200	1 386	99	1 061
SE	18	0.2	1.0	43	69	23	131	17	109
R-B	0.001 < P < 0.005			n.s.			P < 0.001		
A-C	n.s.			n.s.			0.01 < P < 0.05		

$2.0 \pm 0.03 \text{ ml kg}^{-1} \text{ min}^{-1} (\text{mmHg})^{-1}$ ($0.01 < P < 0.05$). The changes in blood pressure and renal blood flow are intimately related to the observed changes in urine flow.

In 4 expts. the effect of vanadate ($0.5 \mu\text{mol kg}^{-1} \text{ min}^{-1}$) on the renal clearance of ¹⁴C-inulin was examined. The results are listed in Table 1 and a typical experiment is illustrated in Fig. 1. In two of the cats urine from both ureters was examined, but in two cats urine from only one ureter was used as bleeding occurred in the other. It will be noticed that vanadate induced severe but reversible fall in ¹⁴C-inulin clearance which corresponded in time to the observed changes in urine production. From Table 1 it appears that, contrary to the marked changes in ¹⁴C-inulin clearance caused by vanadate, there was no significant change in the urine-to-plasma ratio of ¹⁴C-inulin. In these experiments slight but not significant increases in the urinary excretion rate of sodium and potassium occurred after termination of vanadate administration (0.88 ± 0.16 to

1.44 ± 0.49 and 1.00 ± 0.27 to $1.55 \pm 0.44 \mu\text{Eq kg}^{-1} \text{ min}^{-1}$ respectively).

In 1 expt. the effect of vanadate on ¹⁴C-inulin clearance was examined after previous administration of Furosemide (1 mg kg^{-1}). The results are listed in Table 2. It will be seen that, as expected, Furosemide caused a marked increase in urine flow and an equally marked decrease in urine-to-plasma inulin concentration ratio while the clearance of inulin was only slightly depressed. Also under these circumstances did vanadate decrease urine flow whereas the urine-to-plasma ratio of ¹⁴C inulin remained unaltered.

Vanadate and vascular reactions In a series of experiments the effect of vanadate on renal hemodynamics as well as the hemodynamics of other vascular beds were further examined. Doses up to $0.5 \mu\text{mol kg}^{-1}$ were without effect. $0.5 \mu\text{mol kg}^{-1}$ caused an increase in mean arterial blood pressure from 129 ± 8 to $146 \pm 7 \text{ mmHg}$ ($n=11$). $1.0 \mu\text{mol kg}^{-1}$ increased blood pressure from 123 ± 10 to

Table 2. The effect on kidney function of Furosemide 1 mg kg^{-1} followed by vanadate $0.5 \mu\text{mol kg}^{-1} \text{ min}^{-1}$

	Urine flow (mg kg ⁻¹ min ⁻¹)		¹⁴ C-inulin urine ¹⁴ C-inulin plasma		¹⁴ C-inulin clearance (mg kg ⁻¹ min ⁻¹)	
	R	L	R	L	R	L
Control	3.3	3.1	381	390	1 260	1 195
Furosemide	109.4	115.0	9	9	1 030	1 001
Vanadate	0.9	0.6	5	7	5	4

n=6 cats.

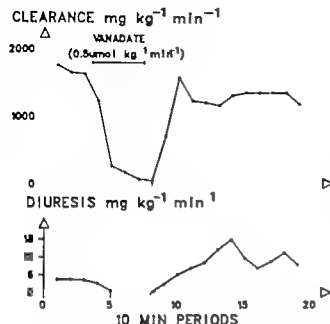


Fig. 1 The effect of vanadate on renal clearance of inulin and on urine flow.

covery period of at least 60 min was allowed, followed by a control period of 1 h during which steady state was obtained. In the experiments with single injections of vanadate, the cat was allowed to recover between the injections.

Analytical procedures. ^{14}C -activity in plasma and urine was measured in a Packard Tricarb Liquid Scintillation Spectrometer as previously described (Kjarrup et al. 1976). Sodium and potassium were determined by flame photometry and chloride by potentiometric titration. Plasma vanadate was determined by a method which takes advantage of the shift in equilibrium binding of ^{51}V vanadate to Na^+/K^+ ATPase upon addition of an aliquot of serum (Hansen 1980).

The same principle was used for determination of a possible influence of chloralose and its degradation product on the binding of vanadate to Na^+/K^+ ATPase.

Calculations. Renal clearance of inulin was calculated as urine flow related to body weight multiplied by urine-to-plasma ratio of ^{14}C inulin. Also blood flow was related to body weight ($\text{ml kg}^{-1} \text{min}^{-1}$) and the unit for pressure was mmHg . Arterial conductance was calculated as mean arterial blood flow divided by mean arterial blood pressure, portal venous conductance as mean portal flow divided by mean portal pressure and mesenteric conductance as mean portal flow divided by the difference between mean arterial and portal pressure. Conductance is thus expressed as $\text{ml kg}^{-1} \text{min}^{-1} (\text{mmHg})^{-1}$. The results are given as mean values \pm S.E. and the effect of vanadate was tested by the method of paired comparisons using the Student's *t*-test.

RESULTS

Vanadate and kidney function. Injection of vanadate in doses up to $1.0 \mu\text{mol kg}^{-1}$ did not affect

urine production whereas $5.0 \mu\text{mol kg}^{-1}$ always decreased urine flow. Higher doses in addition to the effect on kidney function often elicited bradycardia, cardiac arrhythmia and hypotension. In experiments a single injection of $5.0 \mu\text{mol kg}^{-1}$ caused a marked transient fall in urine production from 7.94 ± 0.65 to $2.67 \pm 0.83 \text{ mg kg}^{-1} \text{min}^{-1}$ and after about 40 min urine production reached a peak value of $9.30 \pm 0.74 \text{ mg kg}^{-1} \text{min}^{-1}$ which was not significantly higher than control urine flow. The urinary excretion rate of sodium in the control period and the post injection period at peak urine production was 0.63 ± 0.13 and $0.69 \pm 0.16 \mu\text{Eq kg}^{-1} \text{min}^{-1}$ (*n.s.*) whereas potassium excretion rate increased from 1.87 ± 0.47 to $3.23 \pm 0.36 \mu\text{Eq kg}^{-1} \text{min}^{-1}$ ($0.005 < P < 0.01$).

When given as a continuous infusion vanadate did not cause any change in urine production in dose rates up to $0.1 \mu\text{mol kg}^{-1} \text{min}^{-1}$. Infusions of $0.5 \mu\text{mol kg}^{-1} \text{min}^{-1}$ always decreased urine production and this rate of infusion could be tolerated for up to 60 min. Higher dose rates could only be tolerated for a short period of time and caused cardiovascular disturbances as seen with injections of high doses of vanadate. A few min after the onset of the infusion of $0.5 \mu\text{mol kg}^{-1} \text{min}^{-1}$ urine flow started to fall. It remained low during the infusion which lasted about 70 min and when the infusion was stopped the flow returned gradually within approx. 30 min to a value which was slightly but not significantly higher than the control value (see Larsen et al. 1979, Fig. 1 and Table 1). The mean urine flow in 6 expts. was 7.93 ± 1.07 before, 0.61 ± 0.2 during and $15.94 \pm 3.70 \text{ mg kg}^{-1} \text{min}^{-1}$ after vanadate infusion. The urinary excretion rate of sodium and potassium was 0.79 ± 0.17 and $1.82 \pm 0.74 \mu\text{Eq kg}^{-1} \text{min}^{-1}$ before vanadate infusion and increased slightly after the infusion was stopped to 1.91 ± 0.76 and $3.51 \pm 1.28 \mu\text{Eq kg}^{-1} \text{min}^{-1}$ respectively. The post infusion values were not significantly different from the control values. The excretion rate of sodium and potassium was not measured during the infusion because of the small amount of urine produced.

In four of these experiments mean blood pressure and renal blood flow were also followed. During vanadate infusion mean arterial blood pressure increased from 116 ± 5 to $155 \pm 5 \text{ mmHg}$ ($0.020 < P < 0.025$), renal blood flow decreased from 6.7 ± 1.3 to $2.4 \pm 0.3 \text{ ml kg}^{-1} \text{min}^{-1}$ ($0.070 < P < 0.025$) and renal arterial conductance decreased from 0.058 ± 0.011

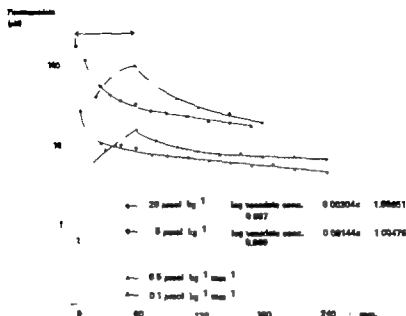


Fig. 3 Plasma concentrations of vanadate-like activity before and following bolus injection or infusion of vanadate.

the renal vascular beds to an increase in conductance in the femoral and carotid vascular beds. The effect of vanadate on blood flow and conductance are illustrated in Fig. 2. The duration of the vascular effect is dose dependent, being about 5 min for the smallest dose and up to 45–60 min for 5.0 $\mu\text{mol kg}^{-1}$.

In an attempt to elucidate the vascular action of vanadate the effect of different vasoactive substances on the vascular response to vanadate was tested in 2–3 cats. However Phenoxybenzamine (5 mg kg^{-1}), Verapamil (10–20 $\mu\text{g kg}^{-1} \text{ min}^{-1}$), Isradipine (1 mg kg^{-1}) or Nifedipine (50 $\mu\text{g kg}^{-1}$) did not change the vascular response to vanadate.

Plasma concentrations of vanadate. In four experiments the plasma concentrations of vanadate after a single injection or continuous infusion of vanadate were followed. The vanadate-like activity in plasma before vanadate was given was in 6 expts. $0.54 \pm 0.03 \mu\text{M}$. The time concentration curves from the experiments are illustrated in Fig. 3. About 60 min after a bolus injection the curve appears to be monoexponential. From this part of the curve half time ($T_{1/2}$) was calculated to 200 min or 150 min for 5 or 20 $\mu\text{mol kg}^{-1}$ respectively. Vanadate was given The solvent space for vanadate in the two experi-

ments was found to be 5% and 50% of body weight, respectively. When comparing the time concentration curves with the observed vascular effects of vanadate, the effects were evoked at plasma vanadate concentrations of about 30 μM .

The effect of vanadate on plasma concentration of Na and K. In 6 expts. with continuous infusion (0.5 $\mu\text{mol kg}^{-1} \text{ min}^{-1}$) vanadate did not change the plasma concentration of Na which was $149 \pm 0.3 \text{ mEq L}^{-1}$ before infusion and $149 \pm 0.5 \text{ mEq L}^{-1}$ at the end of the infusion. In contrast plasma K increased in 8 expts from $5.27 \pm 0.30 \text{ mEq L}^{-1}$ before infusion to $6.40 \pm 0.48 \text{ mEq L}^{-1}$ ($0.005 < P < 0.01$, $n=6$) during vanadate infusion and returned to $5.39 \pm 0.64 \text{ mEq L}^{-1}$ within 1 to 2 h after the infusion was stopped.

Chloralose and vanadate binding. In order to test whether chloralose anaesthesia might interfere with binding of vanadate to Na, K ATPase the effect of chloralose and its degradation products, chloralhydrate and trichlorethanol on the binding of vanadate to the enzyme was tested in 'in vitro' experiments. Enzyme prepared from ox brain was incubated with $5 \cdot 10^{-6} \text{ M}$ Na^{25}V vanadate in the presence and absence of the drugs. Addition of 0.05–0.50 mg ml^{-1} did not significantly change the

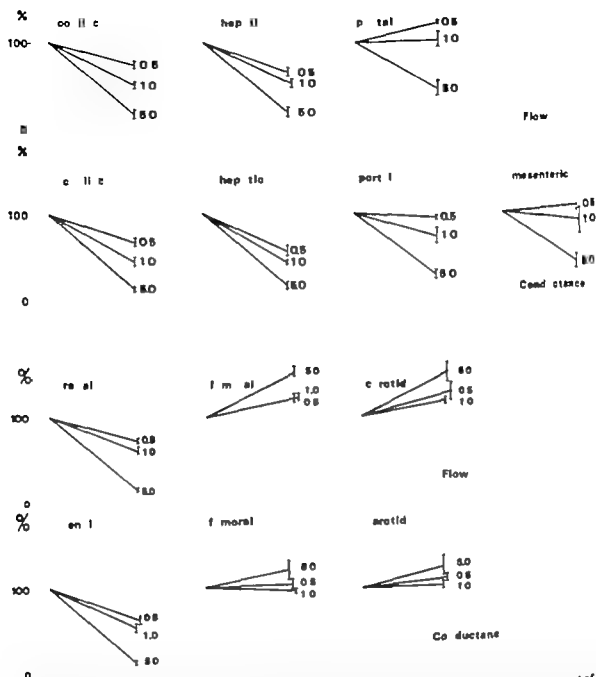


Fig. 2. The effect of vanadate 0.5, 1.0 and 5 $\mu\text{mol/kg}$ on blood flow and conductance expressed in per cent of the control value. In the control state the following values were obtained (mean \pm S.E.). Flows (ml/kg/min): Portal: 49 ± 3.6 , Hepatic: 16 ± 2.5 , Coeliac: 21.2 ± 5.5 , Renal: 6.0 ± 1.8 , Carotid: 6.7 ± 0.7 , Femoral: 4.0 ± 1.3 . Conductances (ml/kg/min (mmHg)): Portal: 4 ± 0.708 , Mesenteric: 0.1 ± 0.04 , Hepatic: 0.133 ± 0.006 , Coeliac: 0.143 ± 0.040 , Renal: 0.049 ± 0.014 , Carotid: 0.052 ± 0.007 , Femoral: 0.034 ± 0.010 .

9 mmHg ($n=12$) and when 5.0 $\mu\text{mol/kg}$ was injected blood pressure increased from 130 ± 8 to 165 ± 9 mmHg ($n=17$). These changes were all highly significant ($P < 0.001$). The rise in blood pressure was not accompanied by significant changes in heart rate.

Portal venous pressure increased from 6.3 ± 0.8 to 8.0 ± 1.2 mmHg ($n=4$) when 0.5 $\mu\text{mol/kg}$ was given.

en and from 7.3 ± 1.5 to 10.7 ± 1.8 mmHg ($n=3$) with 1.0 $\mu\text{mol/kg}$. These changes were not significant, whereas 5.0 $\mu\text{mol/kg}$ caused a significant increase in pressure from 8.0 ± 1.2 to 12.6 ± 1.9 mmHg ($0.001 < P < 0.005$, $n=3$). Vanadate did not increase the pressure in the inferior vena cava.

Conductance also varied with the amount of vanadate given from a marked fall as observed in

ly, this resulted in a reversible reduction in renal flow and a decrease in renal venous outflow and renal venous pressure.

The present experiments have demonstrated that vanadate also constricts other splanchnic vessels. The effect is dose dependent within rather narrow limits, reversible and apparently mediated by a direct effect on the vessel musculature. Simultaneous administration of α - or β -blockers as well as blockers of Ca^{++} -transport did not hinder or modulate the vanadate induced constriction. In contrast to the effect on splanchnic vessels vanadate caused an increase in blood flow in the femoral and carotid artery while the vascular conductance increased or remained unchanged.

Similar vascular changes caused by vanadate were suggested years ago by Jackson from oncometer measurements (1917). Recently Steffen et al. (1979) and Inciarte et al. (1979) reported that intravenous administration of vanadate produced constriction of all components of the skin and skeletal muscle vasculature bed of the dog forelimb as well as a rise in pulmonary resistance. In construction of these experiments Hout et al. (1979) found that vanadate increased tension in the isolated saphenous vein from the dog at a concentration of 10^{-4} M and this effect was not affected by Phentolamine. In the perfused rat liver vanadate at concentrations from 9–50 μM caused dose dependent fall in hepatic vascular conductance (Thomsen & Larsen 1980). Finally it has been shown by Bell et al. (1979) that vanadate caused vasoconstriction in the perfused, irrigated gills of the eel, this effect was completely inhibited by Bayrethine.

It is known that vanadate influences several enzymes (see Sorenson 1979) but the action of vanadate on vascular smooth muscle resembles that of ouabain (Treat et al. 1971; Friedman et al. 1973; Webb & Bohr 1978). It might therefore be expected that the vascular effect is caused by an inhibition of Na, K-ATPase in the vascular smooth muscle. This is supposed to lead to a secondary increase in the intracellular concentration of Ca^{++} with contraction of the muscle cells as a consequence (Schwartz et al. 1973; Blaustein 1977). The results of Hout et al. (1979) are in conflict with this. They found in experiments on the isolated saphenous vein that vanadate did not influence the ouabain-sensitive ^{86}Rb -adenine uptake in vascular smooth muscle which is supposed to be a measure of Na, K-ATPase activity. Furthermore the observed lack of effect of

Ca^{++} -transport blockers on vanadate induced vasoconstriction seems to speak against this hypothesis.

The authors are indebted to Dr H. Hansen, Institute of Physiology University of Aarhus for determination of vanadate. We also thank M. K. Donby and A. Molbeck for skilled technical assistance.

REFERENCES

- BALFOUR, W. E., GRANTHAM, J. J. & GLYNN, I. M. 1978. Vanadate-stimulated natriuresis. *Nature* 275: 768.
- BELL, M. V., KELLY, K. F. & SARGENT, J. R. 1979. Sodium orthovanadate: a powerful vasoconstrictor in the gills of the common eel *Anguilla anguilla*. *J. Mar. Biol. Ass. U.K.* 59: 429–435.
- BLAUSTEIN, M. P. 1977. Sodium ions, calcium ions, blood pressure regulation, and hypertension: reassessment and hypothesis. *Am. J. Physiol.* 232: C165–C173.
- BONVENTRE, J. V., ROMAN, R. J. & LECHENE, C. 1979. Effect of vanadate on renal sodium and water excretion. *Fed. Proc.* 38: 1042.
- CANTLEY, L. C. J. & AISEN, P. 1979. The fate of cytoplasmic vanadium. *J. Biol. Chem.* 254: 1781–1784.
- CANTLEY, L. C. J., JOSEPHSON, L., WARNER, R., YANAGISAWA, M., LECHENE, C. & GUIDOTTI, G. 1977. Vanadate is a potent (Na, K)-ATPase inhibitor found in ATP derived from muscle. *J. Biol. Chem.* 252: 7421–7423.
- CHARNEY, A. N., SILVA, P. & EPSTEIN, F. H. 1975. An in vitro inhibitor of Na-K ATPase present in an adenonucleotriphosphat preparation. *J. Appl. Physiol.* 39: 156–158.
- FRIEDMAN, S. M., NAKASHIMA, M., PALATY, V. & WALTERS, M. K. 1973. Vascular resistance and N, K gradients in the perfused rat-tail artery. *Can. J. Physiol. Pharmacol.* 51: 410–417.
- GRANTHAM, J. J. & GLYNN, I. M. 1979. Renal Na, K ATPase: determinants of inhibition by vanadium. *Am. J. Physiol.* 236: F530–F535.
- HANSEN, O. 1980. Vanadate interaction with the Na, K ATPase. *Basic Res. Cardiol.* 75: 455–459.
- HOUT, S., MULDOON, S., PAMNANI, M., CLOUGH, D. & HADDY, P. J. 1979. Effects of sodium vanadate (Na_2VO_4) on wall tension and N, K pump activity in isolated canine saphenous vein. *Fed. Proc.* 38: 1036.
- INCIARTE, D. J., STEFFEN, R. P., SWINDALL, B. T., JOHNSTON, J. R., DOBBINS, D. E. & HADDY, P. J. 1979. Effects of intravenously infused vanadate on the circulation of the anesthetized dog. *Fed. Proc.* 38: 1036.
- JACKSON, D. E. 1912. The pharmacological action of vanadium. *J. Pharmacol. Exp. Ther.* 3: 477–514.
- JOSEPHSON, L. & CANTLEY, L. C. J. 1977. Isolation of a potent (Na-K)-ATPase inhibitor from striated muscle. *Biochemistry* 16: 4572–4578.
- KRARUP, M., LARSEN, J. A. & MUNCK, A. 1976. Secretin-like choleretic effect of prostaglandins E_1 and E_2 in cats. *J. Physiol.* 254: 813–820.

equilibrium binding level of vanadate established during 60 min of incubation

DISCUSSION

The inhibitory effect of vanadate on Na⁺-K⁺-ATPase (Charney et al 1975, Josephson & Cantley 1977, Cantley et al 1977) has renewed the interest for the biological actions of vanadate. Among the recently discovered effects are the diuretic and natriuretic effects observed in unanesthetized rats (Balfour et al 1978) in which vanadate (approx. 5 µmol/kg given i.v.) increased urine flow and renal sodium excretion rate by 14–16 times. These results were only partly confirmed by Bonventre et al (1979).

The present experiments have demonstrated that vanadate given i.v. in comparable amounts and giving approx. the same plasma concentrations as found by Balfour et al (1978) inhibits urine production in the anesthetized cat. The fall in urine production was reversible and accompanied by marked and similarly reversible constriction of the renal vasculature and fall in renal blood flow.

A simultaneous fall in glomerular filtration rate was seen whereas the urine-to-plasma ratio of ⁵¹Cr-inulin and thus the fractional water excretion rate was not significantly affected by vanadate. This was even the case under conditions where tubular reabsorption and thereby this ratio was markedly reduced by furosemide.

The application of a flowmeter on the renal artery which involves denervation did not affect renal function in the control period or the action of vanadate. This indicates a direct effect of vanadate on vascular smooth muscle.

The observed fall in urine production accompanied by almost unchanged fractional water excretion rate is characteristic for the high degree of glomerulotubular balance which has been found during acute reductions in glomerular filtration rate caused by partial clamping of the renal artery or by other means (see Pitts 1974). The fall in urine production caused by vanadate may therefore be explained by a fall in glomerular filtration rate secondary to renal vasoconstriction and a corresponding fall in glomerular filtration pressure.

This effect is in contrast to the diuretic action in the rat (Bonventre et al 1979) where vanadate was without effect on glomerular filtration rate but caused an increase in fractional water excretion rate

—together with an increase in sodium excretion rate. Their results are consistent with an inhibition of Na⁺-K⁺-ATPase and thereby salt and water reabsorption in the proximal renal tubules although it might be expected that a fall in proximal fluid reabsorption would cause a fall in glomerular filtration rate.

It thus appears that in the cat the effect of vanadate on renal function is dominated by a constriction of renal blood vessels whereas in the rat an inhibition of Na⁺-K⁺-ATPase in the renal proximal tubules is the main action. However, the action of vanadate in the rat seems to be highly dependent on the dose given and the route of administration (Bonventre et al 1979) and this together with an observed increase in blood pressure indicates that vanadate under certain conditions may elicit vasoconstriction eliminating or reducing the diuretic effect of its action on tubular Na⁺-K⁺-ATPase. Likewise it cannot be excluded that vanadate inhibits proximal reabsorption of salt and water also in the cat renal tubules but that this effect is obscured by its marked action on renal vessels. If all nephrons might be urine producing but at a markedly reduced glomerular pressure it may also be that vasoconstriction interferes with the contact of vanadate with Na⁺-K⁺-ATPase and that the local hypoxia produced might favour the reduction of vanadate (+5 oxidation state) to e.g. vanadyl (+4 oxidation state) which is less effective in inhibiting Na⁺-K⁺-ATPase (Cantley & Aisen 1979, Grantham & Glynn 1979).

Although the present results are in contrast to those obtained in rats they are in accordance with experiments on Pentobarbitone-anesthetized dogs as reported by Mills & Newport (1979). They found that infusion of vanadate (0.54 µmol/min) into the renal artery caused hypertension, renal vasoconstriction and a 50–60% reduction in both urine production and glomerular filtration rate. Urinary excretion rate of sodium was not affected but a slight fall in plasma sodium concentration was observed. In the present experiments plasma Na⁺-concentration was not affected by vanadate, whereas a significant rise in plasma K⁺-concentration was observed. This rise can only be partly explained by decreased renal excretion of potassium during infusion of vanadate and may be caused by inhibition of Na⁺-K⁺-ATPase elsewhere. Also Luchini et al (1979) state in a preliminary report that vanadate given i.v. to dogs in doses of 0.2–1.0 µmol

Cerebrovascular sympathetic denervation and blood brain barrier function in conscious rats

HENRIK HARDEBO, LARS EDVINSSON AND BARBRO B. JOHANSSON

Departments of Histology and Neurology, University of Lund, and the Department of Neurology
University of Göteborg, Sweden

HARDEBO J. E., EDVINSSON L. & JOHANSSON B. B. Cerebrovascular sympathetic denervation and blood-brain barrier function in conscious rats. *Acta Physiol Scand* 1980; 110: 375-379. Received 25 Febr. 1980. ISSN 0001-6772. Departments of Histology and Neurology, University of Lund and Department of Neurology, University of Göteborg, Sweden.

Electrical stimulation of the sympathetic nerves to the cerebrovascular bed enables the resistance vessels to better withstand a high blood pressure in terms of blood-brain barrier integrity. Sympathetic denervation could hence be expected to lead to a decrease in cerebrovascular tone and increased vulnerability of the blood-brain barrier. In the present study acute hypertension was induced in conscious unrestrained rats by administration of angiotensin or bicuculline. The albumen leakage into the brain, as studied by Evans blue-albumin and ¹²⁵I labelled human serum albumin, was not enhanced in acutely or chronically sympathectomized rats compared to controls.

Key words. Cervical sympathectomy, acute hypertension, blood-brain barrier, albumin tracers.

The brain vascular bed receives an ample supply of sympathetic adrenergic nerves originating in the superior cervical ganglion (Nielsen & Owman 1967). A reduction of the cerebral blood flow (CBF) by about 10-15% is obtained upon sympathetic nerve stimulation at normotension and normocapnia (for review see Edvinsson and MacKenzie 1977; Heistad et al. 1978; Purves 1978). The sympathetic nervous activity thus lead to an increase in cerebrovascular tone via activation of cerebrovascular alpha-adrenergic receptors (Edvinsson & Owman 1974). There is evidence supporting physiological role of these nerves as reflected in the recent observation that stimulation of the sympathetic trunk in the neck extends the upper limit of autoregulation towards higher blood pressure levels (Bill & Linder 1976; Edvinsson et al. 1976; MacKenzie et al. 1977; MacKenzie et al. 1979).

In acute hypertension the mechanical stress on the cerebral vessels increases the cerebrovascular permeability to proteins (for references see Johansson 1976). Concomitant vasodilatation—as induced by e.g. papaverine, bicuculline, amphetamine or hypercapnia—enhances the extent of blood-brain

barrier (BBB) dysfunction (Johansson 1974; Johansson & Nilsson 1977; Carlsson & Johansson 1978; Hardebo 1980). Stimulation of the sympathetic nerves to the brain vessels enables the resistance vessels to better withstand a sudden blood pressure increase i.e. protein extravasation and flow increment are prevented in acute hypertension (Bill & Linder 1976). An ameliorative effect of sympathetic stimulation is also obtained when acute hypertension is combined with metabolic vasodilatation (Johansson & Lund 1978). Sympathectomy on the other hand, might be expected to result in a reduction in cerebrovascular tone and, possibly, an increased vulnerability—in terms of BBB function—to acute hypertension. This condition is examined in the present study with particular attention to the preexisting vascular tone in the test situation.

MATERIAL AND METHODS

The experiments were performed on 49 conscious male Sprague-Dawley rats (200-300 g b. wt.). A catheter was implanted in the aorta under barbitalurate anaesthesia 2-3 days prior to the experimentation with induction of acute hypertension. Simultaneously the right external jugular

- LARSEN J A THOMSEN O Ø & HANSEN O 1979 Vanadate-induced oliguria in the anesthetized cat. *Acta Physiol Scand* 106: 495-496
- MILLS I H & NEWPORT P A 1979 Renal vasoconstriction, hypertension and fall in urinary osmolality produced by arterial vanadate infusion. *J Physiol* 296: 9-93P
- PITTS R F 1974 Physiology of the kidney and body fluids. Year Book Medical Publ. Inc. Chicago 3rd ed. p. 110
- SCHWARTZ A LINDENMAYER E & ALLEN J C 1975 The sodium-potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. *Pharmacol Rev* 27: 1 pp. 93-101
- SIMONS T J B 1979 Vanadate—a new tool for biologists. *Nature* 281: 337-338
- STEFFEN R P INCIARTE D J SWINDALL T JOHNSTON J & HADDY F J 1979 The effect of vanadate on the dog heart. *Fed Proc* 38: 1440
- THOMSEN O Ø & LARSEN J A 1980 The effect of vanadate on liver hemodynamics and bile production of the perfused rat liver. *Acta Physiol Scand* 106: 4A
- TREAT E ULANO H II & JACOBSON E D 1977 Effects of intra-arterial ouabain on mesenteric and carotid hemodynamics. *J Pharmacol Exp Ther* 171: 144-148
- WEBB R C & BOHR D F 1978 Potassium-induced relaxation as an indicator of Na⁺-K⁺ ATPase activity in vascular smooth muscle. *Blood Vessels* 15: 198-207

Table 2 ¹²⁵I-HSA content in the cerebral cortex, caudate nucleus and cerebellum after acute hypertension induced by $10 \mu\text{g kg}^{-1}$ angiotensin II amide I in awake unestrained rats at various time intervals after cervical sympathectomy (sy-ect) and in controls

For details of the groups, see Table 1. The relative activity is shown as the ratio of activity between brain and blood $\times 100$. Mean values \pm S.E.

Experimental groups		Cortex dx	Caudate nucl dx	Cortex sin	Caudate nucl sin	Cerebellum
Control	7	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.15 ± 0.03
Acute sy-ect dx	6	0.07 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	0.08 ± 0.01
Acute sy-ect sin	8	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.12 ± 0.01
Chronic sy-ect dx (3) (bald 6)	9	0.06 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.10 ± 0.02

is that these nerve fibres participate in the regulation of the cerebral vascular tone, however only to a considerable extent at extreme situations such as acute systemic hypertension. In this situation a supportive function of the sympathetic innervation has been demonstrated in anesthetized animals in terms of ability to maintain both respiratory capacity and blood-brain barrier integrity (see Introduction). A decreased ability to withstand an abrupt increase in intraluminal pressure has been demonstrated (following sympathectomy in lightly anesthetized with nitrous oxide and immobilized by tubocurarine (Edvinsson et al 1971). The high initial mean arterial pressure in these animals (MAP 140–160 mmHg) suggests a stress situation as do high circulating levels of adrenaline and noradrenaline in anesthetized or awake, immobilized animals as compared to freely moving animals (Depoens & Behrnes 1977; Bühler et al 1978; Seavedra et al 1979).

The present results indicate that a tonic influence on the blood-brain barrier function of the sympathetic nerve is minor or absent in conscious unestrained rats subjected to acute hypertension induced by angiotensin II and bicuculline. The initial

sympathetic activity is probably low as compared to anesthetized rats and the increase in blood pressure caused by an injection of a vasoactive drug is not likely to be accompanied by an emotional arousal of a kind that may occur in connection with blood pressure elevations during daily life. It may therefore not seem surprising that sympathectomy did not influence the angiotensin II-induced opening of the barrier in our study. However sympathectomy did even not increase the protein leakage in rats after bicuculline administration. The significantly lower extravasation in sympathectomized rats given bicuculline is likely to be related to the lower maximum blood pressure in these rats (Tables 1 and 3). Whereas the magnitude of the blood pressure increase as well as the maximum pressure level is of importance in acute hypertension per se, the maximum pressure is the main factor responsible for the protein leakage in those experimental models in which hypertension is combined with pronounced cerebral vasodilatation (cf. Johansson & Nilsson 1977).

Epileptic discharge is accompanied by an intense sympathetic activation which is the cause of the blood pressure increase. In a recent study (Møller

Table 3 ¹²⁵I-HSA content in the cerebral cortex, thalamus and cerebellum after injection of 1.2 mg kg^{-1} bicuculline in awake unestrained rats at various time intervals after acute (20–75 min) or chronic (10–12 days) sympathectomy (sy-ect) and in controls

Experimental group		Cortex dx	Thalamus dx	Cortex sin	Thalamus sin	Cerebellum
Control						
Acute sy-ect dx (3), bald (1)	10	0.19 ± 0.02	0.41 ± 0.04	0.20 ± 0.02	0.44 ± 0.04	0.18 ± 0.03
Chronic sy-ect bald	4	0.07 ± 0.01	0.17 ± 0.09	$0.07 \pm 0.02^*$	0.19 ± 0.08	$0.09 \pm 0.02^{**}$
	5	0.08 ± 0.01	$0.09 \pm 0.02^{**}$	$0.09 \pm 0.02^*$	0.11 ± 0.03	$0.10 \pm 0.02^*$

* $P < 0.05$, ** $P < 0.01$ for difference from control group

Table 1 Mean arterial pressure (MAP) before and after a blood pressure increase induced by angiotensin ($20 \mu\text{g kg}^{-1}$) or by bicuculline (1.2 mg kg^{-1}) in rats after acute (4 min–5 h) or chronic (4–10 days) cervic sympathectomy (sy-ect) and in controls

The sympathectomy was performed either by removal of the superior cervical ganglion (gangl) or by section of 1–4 preganglionic nerve (pregangl). PaCO_2 and PaO_2 immediately before the induction of hypertension. Mean value \pm S.E.

Experimental group	n	Initial MAP (mmHg)	Maximum MAP (mmHg)	Δ MAP (mmHg)	PaCO_2 (kPa)	PaO_2 (kPa)
Angiotensin						
Control	7	113 \pm 5	117 \pm 5	64	4.1 \pm 0.1	1.0 \pm 0.1
Acute sy-ect dx (gangl)	6	95 \pm 5	188 \pm 5	93	3.8 \pm 0.1	12.2 \pm 0.2
Acute sy-ect dx (pregangl)	8	110 \pm 3	176 \pm 5	66	3.9 \pm 0.1	13.7 \pm 0.5
Chronic sy-ect dx (3) bilat (6) (pregangl)	9	111 \pm 3	188 \pm 3	77	4.0 \pm 0.1	12.5 \pm 0.2
Bicuculline						
Control	10	117 \pm 5	189 \pm 3	72	4.5 \pm 0.1	1.1 \pm 0.1
Acute sy-ect dx (3) bilat (1) (gangl)	4	101 \pm 4	174 \pm 3	73	4.0 \pm 0.1	12.5 \pm 0.1
Chronic sy-ect bilat (pregangl)	5	118 \pm 6	172 \pm 5	54	4.1 \pm 0.1	12.1 \pm 0.1

vein was cannulated for injection of dyes and tracers except in the animals being subjected to acute sympathectomy at a later step. In these latter rats the external jugular vein was cannulated immediately prior to the sympathectomy. The free ends of the aortic and jugular catheters were exteriorized on the back of the neck.

Sympathectomy. Acute unilateral in a few animals bilateral excision of the superior cervical ganglion was performed under ether anaesthesia. In some experiments the preganglionic nerve trunk (about 5 mm below the ganglion) was cut at various time intervals (4 min to 5 h) before the induction of acute hypertension. Chronic uni- or bilateral sympathectomy was performed 4–12 days before the experimentation.

Induction of acute hypertension. The aortic catheter was connected to a transducer and the resting mean arterial pressure (MAP) was recorded in conscious unanaesthetized rats. Blood gases (PaO_2 and PaCO_2) were repeatedly determined. The following drugs were used for detecting blood-brain barrier opening: ^{125}I HSA (human serum albumin $100 \mu\text{Ci kg}^{-1}$) and Evans blue (1 ml of a 2% solution in saline) which in vivo binds to serum albumin. A rise in MAP was induced by an i.v. bolus injection of either angiotensin II amide (Hypertension CIBA $20 \mu\text{g kg}^{-1}$) or bicuculline (1.2 mg kg^{-1})—a drug that provokes epileptic activity and hemodynamically gives an abrupt rise in blood pressure combined with pronounced cerebral vasodilatation. The resulting BBB dysfunction is exclusively pressure-dependent (Johansson & Nilsson 1977).

3 min after induction of a pressure increase the rats were anesthetized with pentobarbital and the brains were perfused via the left heart ventricle with saline *in situ* for 30 s to remove the blood from the cerebral vessels. After macroscopic examination of Evans blue-albumin extravasation the radioactivity of predetermined brain regions (see Tables 2 and 3) was measured in a gamma counter. The extravasation of ^{125}I HSA was expressed as the percentage ratio of brain and blood activity i.e. $100 \times [\text{CPM/mg brain tissue over CPM/mg blood}]$. Blood sam-

ples were taken immediately before starting the perfusion through the heart. Statistical differences were evaluated with Wilcoxon's rank sum test.

RESULTS

A patchy or dot like extravasation of Evans blue albumin was noted in all rats. Little in rats given angiotensin II and more pronounced particularly in the thalamus of animals given bicuculline. Sympathectomy (unilateral or bilateral) did not significantly alter the amount of extravasation of the tracers ^{125}I HSA or EBA in rats given angiotensin I (Table 2). This was irrespective of whether ganglionectomy was performed acutely (to avoid denervation hypersensitivity) or chronically or whether preganglionic denervation was performed to avoid initial release from the postganglionic neuron caused by manipulation of the ganglion.

DISCUSSION

The density of the cerebrovascular sympathetic plexus is comparable to that of various other peripheral vascular beds and the nerve fibres extend at least in baboon and cat along the cerebrovascular tree down to the level of small microvascular arterioles (Edvinsson & Owman 1977; Cervós Navarro & Matakas 1974). The functional importance of this innervation has been extensively studied during the last few years (for review see Edvinsson & MacKenzie 1977). The major opinion emanating from studies on sympathetic stimulation

- MCCELLER, S. M., REISTAD, D. & MARCUS, M. L. 1979. Effect of sympathetic nerves on cerebral vessels during seizures. *Am J Physiol* 237: H178-H184.
- NELSEN, H. C. & OWMAN, Ch. 1967. Adrenergic innervation of pial arteries related to the circle of Willis. *Acta Otol. Rhinol. Scand* 6: 773-776.
- PURVES, M. J. 1978. Do vasomotor nerves significantly regulate cerebral blood - vessels? *Circ Res* 43: 484-493.
- SAAVEDRA, J. M., KVETNANSKY, R. & KOPIN, I. J. 1979. Adrenaline, noradrenaline and dopamine levels in specific brain stem areas of acutely immobilized rats. *Brain Res* 160: 271-280.

et al 1979) the cervical sympathetic nerve activity was registered before and after injection of bicuculline in cat and shown to increase 3-fold concomitantly with the increase in blood pressure. In agreement with the present results unilateral sympathectomy did not increase the extravasation of protein in the denervated side of the brain. It is possible that the metabolic vasodilatation induced by bicuculline may have masked a constrictory effect of the sympathetic nerves. The fact that sympathetic nerve stimulation significantly decreases bicuculline induced protein leakage in anesthetized rats (Johansson & Lund 1978) does not rule out this possibility as electrical stimulation may not be directly comparable with physiological stimulation.

It may be argued that an abrupt rise in blood pressure as induced presently might precede a reflexory sympathetic vasoconstriction and therefore the present tests would not allow for interpretations of a role for the sympathetic innervation in BBB function. A less rapid rise in pressure did not however potentiate the BBB opening following sympathectomy (unpublished observation) further a lowering in the rate of pressure increase would probably also allow myogenic autoregulation to compensate for the pressure increase.

In spite of all recent evidence that sympathetic nerves do play a role in the regulation of cerebral blood flow and blood-brain barrier integrity at high levels of blood pressure the physiological importance of this mechanism remains to be elucidated.

This study was supported by grants from the Swedish Medical Research Council (84X-737 and 14X-4968) and the Swedish Association Against Heart and Chest Diseases. We wish to thank Professor Christer Owman for valuable discussions.

REFERENCES

- BILL, A. & LINDER, J. 1976 Sympathetic control of cerebral blood flow in acute arterial hypertension. *Acta Physiol Scand* 20: 91-95.
- BÜHLER, H. U., DA PRADA, M., HAEFELY, W. & PICOTTI, G. B. 1978. Plasma adrenaline, noradrenaline and dopamine in man and different animal species. *J Physiol (Lond)* 276: 311-320.
- CARLSSON, C. & JOHANSSON, B. B. 1978. Blood-brain barrier dysfunction after amphetamine administration in rats. *Acta Neuropathol (Berl)* 41: 125-129.
- CERVÓS-NAVARRO, J. & MATAKAS, F. 1974. Electron-microscopic evidence for innervation of intracerebral arterioles in the cat. *Neurology (Milneap)* 24: 282-286.
- DA PRADA, M., PICOTTI, G. B., CARRUBA, M. O. & HAEFELY, W. E. 1979. Plasma catecholamine, metanephrine and p-octopamine levels, stress- and drug-induced changes in rat. In: *Catecholamine: Basic and clinical frontiers* (ed. E. Usdin, J. J. Ko and J. Barchas), pp. 913-917. Pergamon Press, New York.
- DEPOCAS, F. & BEHRENS, W. A. 1977. Effects of handling, decapitation, anesthesia and surgery on plasma noradrenaline levels in the white rat. *Can J Physiol Pharmacol* 55: 12-19.
- EDVINSSON, L. & MACKENZIE, E. T. 1977. Autoregulation mechanisms in the cerebral circulation. *Pharmacol Rev* 28: 773-733.
- EDVINSSON, L. & OWMAN, Ch. 1977. Sympathetic innervation and adrenergic receptors in intracerebral cerebral arterioles of baboon. *Acta Neurol Scand* 56, Suppl. 64: 304-305.
- EDVINSSON, L., HARDEBO, J. E. & OWMAN, Ch. 1977. Influence of the cerebrovascular sympathetic innervation on regional flow, autoregulation and blood-brain barrier function. In: *Cerebral vascular disease: muscle and its control*. Ciba Found Symp 56 (Lanc), pp. 69-95.
- EDVINSSON, L., OWMAN, Ch. & SIESJÖ, B. K. 1977. Physiological role of cerebrovascular sympathetic nerves in the autoregulation of cerebral blood flow. *Brain Res* 117: 519-533.
- HARDEBO, J. E. 1980. Vasodilatation augments blood-brain barrier opening induced by a hypertensive insult. *Blood Vessels*. In press.
- HEISTAD, D. D., MARCUS, M. L. & GROSS, P. J. 1978. Cerebral vascular responses to electrical stimulation of sympathetic pathways in dog, cat and monkey: evidence for an important species difference. *Am J Physiol* 235: H544-H552.
- JOHANSSON, B. B. 1974. Blood-brain barrier dysfunction in acute arterial hypertension after papeaverine induced vasodilatation. *Acta Neurol Scand* 50: 361-370.
- JOHANSSON, B. B. 1976. Brain barrier pathology in acute arterial hypertension. *Adv Exp Med Biol* 69: 517-527.
- JOHANSSON, B. B. & LUND, S. 1978. Effect of sympathetic stimulation on the blood-brain barrier dysfunction induced by amphetamine and by epileptic seizures. *Acta Physiol Scand* 104: 281-286.
- JOHANSSON, B. B. & NILSSON, B. 1977. The pathophysiology of the blood-brain barrier dysfunction induced by severe hypercapnia and by epileptic brain activity. *Acta Neuropathol (Berl)* 38: 153-158.
- MACKENZIE, E. T., McGEORGE, A. P., GRAHAM, D. I., FITCH, L., EDVINSSON, L. & HARPER, A. M. 1977. Breakthrough of cerebral autoregulation and the sympathetic nervous system. *Acta Neurol Scand* Suppl. 64: 36-49.
- MACKENZIE, E. T., McGEORGE, A. P., GRAHAM, D. I., FITCH, L., EDVINSSON, L. & HARPER, A. M. 1979. Effects of increasing arterial pressure on cerebral blood flow in the baboon. Influence of the sympathetic nervous system. *Pharmacol Arch* 378: 189-193.

Dissociation of electrical and mechanical activity caused by vibrations in the spontaneously active smooth muscle of the rat portal vein

A. SÖQVIST and B. LJUNG

Department of Physiology, University of Göteborg, Sweden

SÖQVIST A. & LJUNG B. Dissociation of electrical and mechanical activity caused by vibrations in the spontaneously active smooth muscle of the rat portal vein. *Acta Physiol Scand* 1980, 110: 381-384. Received 5 March 1980. Department of Physiology, University of Göteborg, Box 33031, S-40033 Göteborg, Sweden.

The effects of vibrations on the electrical membrane discharge and on the contractile force of the spontaneously active smooth muscle of isolated rat portal vein were studied. The electrical activity was recorded extracellularly and quantitatively related to the mean active force. Sinusoidal vibrations (40 Hz, 2.5–3.0% tissue length peak to peak) applied in the longitudinal direction of the smooth muscle, caused prompt and reversible reduction of active force but neither the pattern of the phasic contractions nor the electrical membrane discharge was altered. The degree of inhibition of mechanical activity increased with vibration amplitude, activity being 30% of control at vibration amplitude of $12 \pm 4\%$ (mean \pm S.D., $n=8$). It is concluded that the induced length changes caused prompt dissociation between electrical membrane discharge and mechanical force development in the vascular smooth muscle. This finding adds support to the previously forwarded hypothesis that vibrations cause inhibition of contracting muscle by direct action on the contractile process.

Key words: Vascular smooth muscle, portal vein, electrical activity, contractile force, vibrations.

Vibrations have been found to reduce active force development in isolated vascular smooth muscle (Ljung & Sverrisson 1975, 1980). There is indirect evidence that this inhibition of active force in smooth muscle is a direct action by the imposed length changes on the contractile machinery, possibly due to an increased rate of detachment of actin-myosin cross-links (for reference see Ljung & Sverrisson 1980) as originally suggested for skeletal muscle (Joyce, Rack & Westbury 1969).

In the present experiments, the effects of longitudinal vibrations on the spontaneously active smooth muscle of the rat portal vein have been quantitated in terms of electrical discharge of the membrane and compared to the mechanical force output. The method implies a correlation between the integrated electrical and mechanical activities, respectively, when recorded by a method analogous to that used by Ljung & Stage (1970). The results demonstrate that a dissociation between electrical and mechanical activity occurs under the influence of vibrations.

MATERIAL AND METHODS

Isolated portal veins from male rats of the Sprague-Dawley strain, weighing 200–250 g were studied. Under brief ether anaesthesia the rats were sacrificed and the portal vein was carefully dissected free of surrounding tissue, tied at both ends with fine silk and isolated. It was mounted vertically in an organ bath filled with modified Krebs solution at 38°C. The lower end was firmly attached to submerged isometric transducer with compliance of 0.2 mm/N. The transducer utilized semiconductor element (AE 803P, Aikens Electronics, Norway). The upper end was tied to steel rod extending from the core of an electromagnetic vibrator (Mod 201, Ling Dynamic Systems Ltd, Great Britain).

The vein was stretched to resting passive force of 4 mN and left to accommodate for at least 1 h before the experiment was started. The tissue lengths at 4 mN passive force ranged between 3.0–5.4 mm when measured by means of dissection microscope equipped with micrometer eye piece.

The electromagnetic vibrator moved the steel rod in the vertical direction, i.e. it imposed length changes in the direction of the smooth muscle cells of the heavy longitudinal outer media layer of the portal vein (cf. Johansson et al. 1970). An in-built photo-electric device

Dissociation of electrical and mechanical activity caused by vibrations in the spontaneously active smooth muscle of the rat portal vein

A. SJÖQVIST and B. LJUNG

Department of Physiology, University of Göteborg, Sweden

SJÖQVIST A. & LJUNG B. Dissociation of electrical and mechanical activity caused by vibrations in the spontaneously active smooth muscle of the rat portal vein. *Acta Physiol Scand* 1980; 110: 381-384. Received 5 March 1980. Department of Physiology, University of Göteborg, Box 33031, S-40033 Göteborg, Sweden.

The effects of vibrations on the electrical membrane discharge and on the contractile force of the spontaneously active smooth muscle of isolated rat portal vein were studied. The electrical activity was recorded extracellularly and quantitatively related to the mean active force. Sinusoidal vibrations (40 Hz, 2.5-3.0% tissue length peak to peak), applied in the longitudinal direction of the smooth muscle, caused prompt and reversible reduction of active force but neither the pattern of the phasic contractions nor the electrical membrane discharge was altered. The degree of inhibition of mechanical activity increased with vibration amplitude, activity being 50% of control at vibration amplitude of $12 \pm 4\%$ (mean \pm S.D., $n=8$). It is concluded that the reduced length changes caused prompt dissociation between electrical membrane discharges and mechanical force development in the vascular smooth muscle. This finding adds support to the previously forwarded hypothesis that vibrations cause inhibition of contracting muscle by direct action on the contractile process.

Key words. Vascular smooth muscle, portal vein, electrical activity, contractile force, vibrations.

Vibrations have been found to reduce active force development in isolated vascular smooth muscle (Ljung & Sverrisson 1975, 1980). There is indirect evidence that this inhibition of active force in smooth muscle is a direct action by the imposed length changes on the contractile machinery, possibly due to an increased rate of detachment of actin-myosin cross-links (for reference see Ljung & Sverrisson 1980) as originally suggested for skeletal muscle (Joyce, Rack & Westbury 1969).

In the present experiments the effects of longitudinal vibrations on the spontaneously active smooth muscle of the rat portal vein have been quantitated in terms of electrical discharge of the membrane and compared to the mechanical force output. The method implies a correlation between the integrated electrical and mechanical activities, respectively, when recorded by a method analogous to that used by Ljung & Stage (1970). The results demonstrate that a dissociation between electrical and mechanical activity occurs under the influence of vibrations.

MATERIAL AND METHODS

Isolated portal veins from male rats of the Sprague-Dawley strain, weighing 200-250 g were studied. Under brief ether anaesthesia the rats were sacrificed and the portal vein was carefully dissected free of surrounding tissues, tied at both ends with fine silk and isolated. It was mounted vertically in an organ bath filled with modified Krebs solution at 38°C. The lower end was firmly attached to a submerged isometric transducer with compliance of 0.2 mm/N. The transducer utilized a semiconductor element (AE 803P, Alkerm Electronics, Norway). The upper end was tied to a steel rod extending from the core of an electromagnetic vibrator (Mod 201, Ling Dynamic Systems Ltd, Great Britain).

The vein was stretched to a resting passive force of 4 mN and left to accommodate for at least 1 h before the experiment was started. The tissue lengths at 4 mN passive force ranged between 3.0-5.4 mm when measured by means of a dissection microscope equipped with a micrometer eye piece.

The electromagnetic vibrator moved the steel rod in the vertical direction, i.e. it imposed length changes in the direction of the smooth muscle cells of the heavy longitudinal outer media layer of the portal vein (cf. Johansson et al. 1970). An in-built photo-electric device

Effects of vibrations

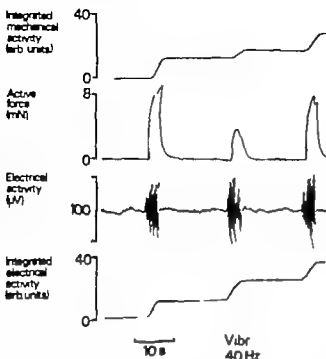


Fig. 1. Tracings of electrical and mechanical spontaneous activity of the rat portal vein. Three consecutive bursts of electrical discharge and associated contractions are seen. Vibrations (40 Hz, 8% tissue length) were applied for 10 s ('Vibr') during which period the middle contraction wave occurred. Note that the contraction amplitude but not the electrical discharge was reduced. Neither was time interval between contractions altered.

registered the position of the core of the vibrator and a potential proportional to the displacement was generated (1 μ V/mm displacement) and fed into a control amplifier (L. Stage unpublished). The control amplifier was connected to the core of the vibrator and to an input signal and forced the displacement signal to follow the input signal very closely. Both signals were continuously monitored on a dual beam oscilloscope. This control system made it possible to induce exact length changes which followed the predetermined characteristics of the input signal with regard to wave form, frequency and amplitude. In the present set of experiments the input signal was sinusoidal with a frequency of 40 Hz and varying amplitudes. Adjustments were made so that the mean passive tension remained constant during the vibrations.

The electrical activity was recorded between an exploring extracellular electrode and a reference electrode. The silver recording electrode was connected to the tissue via a glass tube with a small tip (inner diameter about 0.1 mm). It was filled with Krebs solution and an Ag-AgCl wire was inserted into the tube. The tip of the capillary bridge was placed against the adventitial surface of the portal vein. The reference electrode was an Ag-AgCl wire connected to the bath via an agar-saline bridge. The electrodes were connected via capacitors to a polygraph (Gnost preamplifier mod 7P1E). The force transducer was

connected to the polygraph for isometric recording of active (spontaneous) force developed by the longitudinal smooth muscle layer of the portal vein. The active force produced was integrated over 1 min intervals. The electrical recording was rectified and integrated simultaneously on a separate channel of the polygraph.

The modified Krebs solution used had the following composition in mM: NaCl 120, KCl 4.73, CaCl₂ 2.4, MgCl₂ 1.19, NaHCO₃ 15.5, KH₂PO₄ 1.19, glucose 11.5. It was continuously bubbled with 4% CO₂ in O₂.

Statistic. Two-tailed Student's *t* test was used for calculation of the significance of displacement from control values.

RESULTS

In the control situation the longitudinal smooth muscle of the rat portal veins displayed the typical activity pattern of phasic spontaneous contractions at a rate of 1–3 contractions per min with intervening periods of complete relaxation to the baseline of passive force. Associated with each contraction a burst of action potentials was recorded, the onset of which slightly preceded the development of active force. Fig. 1 illustrates an experimental sequence, where tracings of the electrical and the mechanical activities respectively of a spontaneous contraction in the control situation are shown. The second contraction was recorded during exposure to sinusoidal vibrations (40 Hz, 8% peak to peak amplitude of tissue length) and a subsequent third contraction recorded after cessation of vibrations. In addition to the recordings of active force and electrical discharge the integrals of each of these activities (see methods) are shown in separate tracings of Fig. 1. It is apparent that the vibrations selectively reduced the amplitude of the contraction whereas the duration of the contraction and the interval between the three contractions remained unchanged. Furthermore the electrical discharge was not altered. In order to study the relative effect of vibrations on electrical and mechanical activity respectively the integrated values obtained over one min periods were averaged during a 15 min control period and during subsequent 5 min periods of vibrations at increasing vibration amplitudes. At the end of the vibration exposures the control activity was again quantitated. In Fig. 2 the averaged integrated values of the electrical and mechanical activity during exposure to vibrations at 40 Hz and of the amplitudes indicated (% tissue length, peak to peak) have been expressed as a percentage of the mean integrated electrical and mechanical activity during

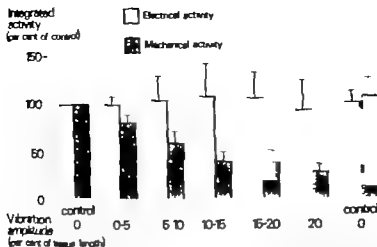


Fig. Effects of vibrations (40 Hz) at graded amplitudes on integrated electrical discharge (open bars) and on integrated active force (hatched bars) of the spontaneously active longitudinal muscle of the rat portal vein. In each experiment the mean activities recorded during 5 one minute periods of vibration exposure at given vibration amplitude were expressed as percentage of the averaged 15 one minute period in the (initial) control situation. Mean \pm S.D. from 8 experiments.

spontaneous vibrations at 40 Hz and of the amplitudes indicated (% tissue length, peak to peak) have been expressed as a percentage of the mean integrated electrical and mechanical activity over the total 15 min control period. The integrated mean active force was diminished to an extent which depended on vibration amplitude ($P < 0.05$ in all groups) whereas the integrated electrical activity was not significantly altered ($P > 0.05$ for all groups). The vibration amplitude which at 40 Hz would cause 50% reduction in mean active force (as determined by interpolation in the individual experiment, see methods) amounted to $1 \pm 4.4\%$ peak to peak of total tissue length (mean \pm S.D.).

During the second control period following cessation of vibrations, neither electrical nor mechanical activity was significantly different from the initial control values (Fig. 2).

DISCUSSION

The vibrations applied in the longitudinal direction of the spontaneously contracting isolated smooth muscle of the rat portal vein reduced the developed active force while the quantitated electrical discharge and the phasic contractile pattern remained unaltered. The force output decreased with increasing amplitude of the applied oscillating length changes in the range 2.5 to 30% of the tissue length.

This confirms previous findings in vascular smooth muscle (Ljung & Sivertsson 1975) as well as in isolated myocardium (Vokas, Sivertsson & Ljung 1978).

In the present experiments the electrical membrane discharge was recorded extracellularly by means of a saline filled large glass electrode. This volume conductor recording system measured bursts of compound impulse activity in the neighbourhood of the electrode tip (cf. Ljung & Stage 1970). During well synchronized smooth muscle activity which normally prevails in homogeneous sections of the longitudinal muscle layer of the rat portal vein the bursts of action potentials will spread throughout the entire muscle. Thus, recorded electrical activity can be considered to reflect the total electrical discharge in the longitudinal smooth muscle layer of the portal vein (cf. Ljung & Stage 1970). The rectified AC signal has been quantitated by means of integration over 1 min periods. In the control situation there seemed to be a linear relationship between the electrical activity and the mechanical activity quantitated as the integral of the force signal obtained over the same 1 min period. When vibrations were applied to the tissue no apparent artifacts were induced in the electrical recording (Fig. 1) and the smooth muscle maintained the same rhythm of contractions. Thus, our finding of unchanged electrical activity but

graded reductions of mechanical activity during vibrations at varied amplitudes demonstrates that the longitudinal oscillations cause a selective interference with the mechanical force generation of the smooth muscle. Joyce, Rack & Westbury (1969) proposed that in skeletal muscle vibrations increased the rate of detachment of actin-myosin cross-links and the present results strongly indicate that this is also true of smooth muscle (see Ljung & Sivertsson 1980).

On the other hand it is well known that in some situations length alterations induce marked changes in electrical discharge and associated changes in mechanical activity. Johansson & Mellander (1975) have shown that slow elongation of the portal vein (0.7 cm muscle length/s for 2 min) caused increased spike discharge and contraction rate whereas slow passive shortening caused decreased electrical and mechanical activity. Cyclic slow stretches and shortenings have been found to make the phasic contractions cluster during the periods of increasing length and to become rarefied during the shortening periods. As the frequency of such cyclic lengthening and shortening increases the membrane effects no longer become manifest in the rat portal vein since the inhibitory effect will apparently counteract the excitatory effect (Sjöqvist & Ljung to be published). However, Ohhashi *et al.* (1979) have reported that "microvibrations" (1–80 Hz, amplitudes less than 1% tissue length) may evoke phasic contractions at an increased rate after a latency of about 1 min in smooth muscle of the ureter and portal vein of the dog. In our experience such an effect does not occur in the isolated rat portal vein unless experimental period extends to 6–8 h. The membrane stability of the smooth muscle seems to decrease progressively in the course of an *in vitro* experiment and thus the membrane sensitivity to stretch is increased. Possibly such changes in the membrane properties may occur more rapidly in the larger isolated smooth muscle specimens studied by Ohhashi *et al.* (1979) than in the thin-walled rat portal vein preparation.

The biological relevance of vibrations to vascular smooth muscle in the physiological situation is not clear. In a recent review Dobrin (1978) reports that with each cardiac cycle conduit arteries undergo 8–10% oscillation in external diameter or about 15% oscillation in internal diameter. The basal frequency of such oscillation would be the same as the heart rate which seems to be in the lower range of

frequencies which may exert significant inhibitory effects. However, the pressure curve is not sinusoidal and many harmonics with higher frequencies but lower amplitudes are present. It remains to be clarified whether their amplitudes are high enough to reduce active force generation.

Under occupational conditions such as work with vibrating tools and in pathological conditions such as arterial stenosis vibrations of considerable frequency and amplitude may occur locally. Such vibrations seem likely to interfere with the contraction process in the vascular wall as previously suggested (Sivertsson & Ljung 1976).

Thanks are due to Ms Ann Kjellstedt for technical assistance, to Mr Lars Stage for construction of electronic equipment and to Ms Lena Ziden for secretarial aid.

Supported by the Swedish Medical Research Council (Grant No. 3884) and by AB Hjälc.

REFERENCES

- DOBRIN P. B. 1978. Mechanical properties of arteries. *Physiol Rev* 58: 397–460.
- JOHANSSON B. & MELLANDER S. 1975. Static and dynamic components in the vascular myogenic response to passive changes in length as revealed by electrical and mechanical recordings from the rat portal vein. *Circ Res* 36: 76–83.
- JOHANSSON B., LJUNG B., MALMFORS, T. & OLSON L. 1970. Prejunctional supersensitivity in the rat portal vein as related to its pattern of innervation. *Acta Physiol Scand Suppl.* 349: 1–16.
- JOYCE, G. C., RACK, P. M. H. & WESTBURY, D. R. 1969. The mechanical properties of cat soleus muscle during controlled lengthening and shortening movements. *J Physiol (Lond.)* 201: 461–74.
- LJUNG B. & SIVERTSSON R. 1974. Vibrations-induced inhibition of vascular smooth muscle contraction. *Blood Vessels* 1: 38–52.
- LJUNG B. & SIVERTSSON R. 1980. The inhibitory response of vascular smooth muscle to vibrations. In: *Vascular neuroeffector mechanisms* (ed. J. A. Bravil *et al.*). Raven Press, New York: 107–121.
- LJUNG B. & STAGE L. 1970. Adrenergic excitatory influences on initiation and conduction of electrical activity in the rat portal vein. *Acta Physiol Scand* 80: 171–81.
- OHASHI T., AZUMA T. & SAKAGUCHI M. 1979. Effect of microvibration on activity of ureteral and portal smooth muscles. *Am J Physiol* 236(5): C19–C20.
- SIVERTSSON R. & LJUNG B. 1976. Vibration-induced changes in vascular tone. *Acta Chir Scand Suppl.* 463: 70–.
- VUKAS M., SIVERTSSON R. & LJUNG B. 1974. Inhibitory effects of vibrations on contractility of isolated rabbit papillary muscle. *Scand J Clin Lab Invest* 38: 415–19.

Characterization of contractions in mechanically disaggregated myocardial cells from the rat

H. LEHTO and R. TIRRI

Laboratory of Animal Physiology, Department of Biology, University of Turku, Finland

LEHTO H. & TIRRI R. Characterization of contractions in mechanically disaggregated myocardial cells from the rat. *Acta Physiol Scand* 1980, 110: 385-389. Received 10 March, 1980. ISSN 0001-6772. Laboratory of Animal Physiology, Department of Biology, University of Turku, Finland.

Myocardial cell groups, mechanically disaggregated from the rat heart, beat spontaneously in a medium which resembles intracellular fluid. The cells initially functioning in an uncoordinated way i.e. each cell in its own rhythm gradually become coordinated as a function of time. After 90 min of incubation at 15°C all the cell groups were functioning in a coordinated way. The initiation of coordination was temperature-dependent. Lowering the temperature from 15°C to 10°C significantly delays the coordination: only 75% of the cell groups being coordinated after 90 min. The contraction wave in coordinated cell groups was very slow: $90 \pm 7 \mu\text{m/s}$ at 20°C, $100 \pm 6 \mu\text{m/s}$ at 30°C and $100 \pm 7 \mu\text{m/s}$ at 35°C. The velocity of contraction waves was not significantly temperature-dependent, suggesting a nonenzymatical type of propagation. It also seems too slow to be an electrical event. Apparently the contraction waves are propagated by diffusion of Ca^{2+} . The addition of caffeine to the medium hastened the coordination of contractions but Na-azide strongly inhibited it. The results indicate that the sarcoplasmic reticulum functions in the coordination of contractions, probably by releasing Ca^{2+} for the activation of the myofilaments.

Key words. Rat, myocardial cells, *in vitro* function

The contraction of a myocardial cell is initiated by an action potential, the depolarization of sarcolemma. The binding of Ca^{2+} with the troponin C terminates the series of events preceding the contraction. The excitation-contraction coupling process is still partially unclear, especially concerning where and by what mechanism Ca^{2+} is released into this system. Two possible mechanisms have been suggested: a Ca^{2+} -induced release of Ca^{2+} from the sarcoplasmic reticulum (Fabiato & Fabiato 1975, 1977) and depolarization-induced release of Ca^{2+} from the sarcoplasmic reticulum (Endo 1977, Thoenen & Endo 1975). On the other hand, there are also suggestions that the major source of coupling Ca^{2+} is external to the sarcolemma and that this extracellular Ca^{2+} moves nonelectrogenically during excitation-contraction coupling (Langer 1976).

A technique has been developed for isolating myocardial cells so that single cells are able to contract rhythmically (Bloom 1970, 1971). The cells function in an incubation medium which resembles intracellular fluid. The cells are

suggested to beat by means of extracellular Ca^{2+} , the sarcolemma being in these circumstances highly permeable to divalent cations (Bloom & Mulholland 1976). Ca^{2+} -influx covaries with contraction frequency in contrast to Ca^{2+} -efflux, which does not (Bloom 1978).

Using Bloom's technique we (Pelkonen & Tirri 1977) found that mechanically disaggregated cell groups from the rat ventricular myocardium can beat in an uncoordinated way i.e. each cell at its own rhythm, or in a coordinated way where one cell serves as a pacemaker. The aim of this study is to further characterize the function of these isolated cell groups and especially to attempt to find out the role of Ca^{2+} in the contraction and relaxation processes.

MATERIALS AND METHODS

Preparation of cell groups

Sprague-Dawley rats of both sexes were used at the age of 7-14 days. This age-group was chosen because we had

graded reductions of mechanical activity during vibrations at varied amplitudes demonstrates that the longitudinal oscillations cause a selective interference with the mechanical force generation of the smooth muscle. Joyce, Rack & Westbury (1969) proposed that in skeletal muscle vibrations increased the rate of detachment of actin-myosin cross links and the present results strongly indicate that this is also true of smooth muscle (see Ljung & Sivertsson 1980).

On the other hand it is well known that in some situations length alterations induce marked changes in electrical discharge and associated changes in mechanical activity. Johansson & Mellander (1975) have shown that slow elongation of the portal vein (0.7% muscle length/s for 2 min) caused increased spike discharge and contraction rate whereas slow passive shortening caused decreased electrical and mechanical activity. Cyclic slow stretches and shortenings have been found to make the phasic contractions cluster during the periods of increasing length and to become rarefied during the shortening periods. As the frequency of such cyclic lengthening and shortening increases the membrane effects no longer become manifest in the rat portal vein since the inhibitory effect will apparently counteract the excitatory effect (Sjöqvist & Ljung to be published). However Ohhashi et al (1979) have reported that "microvibrations (1-80 Hz amplitudes less than 2% tissue length) may evoke phasic contractions at an increased rate after a latency of about 1 min in smooth muscle of the ureter and portal vein of the dog. In our experience such an effect does not occur in the isolated rat portal vein unless experimental period extends to 6-8 h. The membrane stability of the smooth muscle seems to decrease progressively in the course of an in vitro experiment and thus the membrane sensitivity to stretch is increased. Possibly such changes in the membrane properties may occur more rapidly in the larger isolated smooth muscle specimens studied by Ohhashi et al (1979) than in the thin walled rat portal vein preparation.

The biological relevance of vibrations to vascular smooth muscle in the physiological situation is not clear. In a recent review Dobrin (1978) reports that with each cardiac cycle conduit arteries undergo 8-10% oscillation in external diameter or about 15% oscillation in internal diameter. The basal frequency of such oscillation would be the same as the heart rate which seems to be in the lower range of

frequencies which may exert significant inhibitory effects. However the pressure curve is not sinusoidal and many harmonics with higher frequencies but lower amplitudes are present. It remains to be clarified whether their amplitudes are high enough to reduce active force generation.

Under occupational conditions such as work with vibrating tools and in pathological conditions such as arterial stenosis vibrations of considerable frequency and amplitude may occur locally. Such vibrations seem likely to interfere with the contraction process in the vascular wall as previously suggested (Sivertsson & Ljung 1976).

Thanks are due to Ms Ann Kjellstedt for technical assistance to Mr Lars Stage for construction of electronic equipment and to Ms Lena Zaden for secretarial aid.

Supported by the Swedish Medical Research Council (Grant No. 3884) and by AB Hälske.

REFERENCES

- DOBRIN P B 1978 Mechanical properties of arteries. *Physiol Rev* 58: 397-460.
- JOHANSSON B & MELLANDER S 1975 Static and dynamic components in the vascular myogenic response to passive changes in length as revealed by electrical and mechanical recordings from the rat portal vein. *Circ Res* 36: 76-83.
- JOHANSSON B, LJUNG B, MALAIPORS T & OLSON L 1970 Prejunctional hypersensitivity in the rat portal vein as related to its pattern of innervation. *Acta Physiol Scand Suppl* 349: 5-11.
- JOYCE G C, RACK P M H & WESTBURY D R 1969 The mechanical properties of cat soleus muscle during controlled lengthening and shortening movements. *J Physiol (Lond)* 204: 461-74.
- LJUNG B & SIVERTSSON R 1975 Vibration-induced inhibition of vascular smooth muscle contraction. *Blood Vessels* 12: 38-52.
- LJUNG B & SIVERTSSON R 1980 The inhibitory response of vascular smooth muscle to vibrations. In: *Vascular neuroeffector mechanisms* (ed J A Bevan et al). Raven Press, New York: 207-212.
- LJUNG B & STAGE L 1970 Adrenergic excitatory influences on initiation and conduction of electrical activity in the rat portal vein. *Acta Physiol Scand* 80: 131-41.
- OHHASHI T, AZUMA T & SAKAGUCHI M 1979 Effect of microvibration on activity of ureteral and portal smooth muscles. *Am J Physiol* 236(5): C197-C201.
- SIVERTSSON R & LJUNG B 1976 Vibration-induced changes in vascular tone. *Acta Chir Scand Suppl* 465: 20-22.
- VUKAS M, SIVERTSSON R & LJUNG B 1978 Inhibitory effects of vibrations on contractility of isolated rabbit papillary muscle. *Scand J Clin Lab Invest* 38: 415-19.

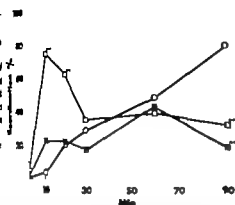


Fig. 2 The effect of caffeine on the coordination of the cell groups incubated in HM at 10°C. At every incubation time (minutes) the percentage of coordinated cell groups (ordinate) calculated in the caffeine experiments, 16–19 cell groups were used at every incubation time, and 23–35 control experiments at every incubation time. The caffeine concentrations were 10^{-4} M (□—□) and 2×10^{-4} M (○—○). The points marked with asterisks differ significantly from the controls ($P < 0.01$).

incubation temperature was 10°C. The difference in coordination in these two temperatures was statistically significant ($P < 0.05$).

Effect of caffeine

Caffeine (10^{-4} M), added to the incubation medium at 10°C, accelerated the appearance of coordination (Fig. 2). After 10 and 20 min of incubation the differences from the control groups in coordination were statistically significant ($P < 0.001$). At 30 and 60 min of incubation, however, there were no differences from the control groups, and at 90 min caffeine had even significantly delayed the coordination ($P < 0.005$). To test the concentration of caffeine, a study slightly stimulated the coordination but later had an inhibitory effect on it. In many cases, however, when incubated for more than 10 min, caffeine caused a permanent partial contracture in cells and this made the observation concerning the coordination more difficult. With still stronger caffeine concentration (5×10^{-4} M) this type of permanent contracture started to soon (in 5–10 min), that the allocation of fraction into the coordinated or uncoordinated type as impossible.

Fig. 3 shows some examples of the experiments where contractions in the cell group were registered with the chart-recorder connected to the photomultiplier. Fig. 3 A is an original recording of con-

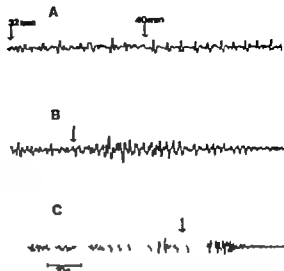


Fig. 3 (A) The coordination of contractions registered with the chart-recorder. The cell group beating in HM gradually becomes coordinated, which can be seen as larger and regular-shaped wave on the right. (B and C) The effect of caffeine on the uncoordinated contractions. The addition of caffeine is marked with an arrow. The caffeine concentrations were 1 mM (B) and 2 mM (C).

tractions from a cell group incubated for 30 min at 15°C. The coordinated contractions are seen as larger regular-shaped waves on the right, which coincided with the coordinated contractions as watched on the microscope. Fig. 3 B shows a recording of contractions when caffeine (1 mM) was added into the examination chamber after 4 min of incubation as indicated by an arrow. Caffeine changes the uncoordinated contractions to the coordinated type, but after some minutes of incubation with caffeine the volume of contractions starts to decrease. A similar effect can be seen using a larger concentration (2 mM) of caffeine (Fig. 3 C) when the depressing effect with a complete contracture follows the coordination in about 10 s.

Effect of azide. Fig. 4 shows the effect of NaN₃ (mitochondrion poison), on the coordination of contractions. It seems that 5 mM NaN₃ strongly inhibited the coordination in the cell groups. After incubating with azide for 60 and 90 min the number of coordinated cell groups was significantly less than in the pure HM ($p < 0.01$). In an additional test series using 50 mM NaN₃ not a single coordinated contraction was found with the uncoordinated contractions, although weakened, still continuing.

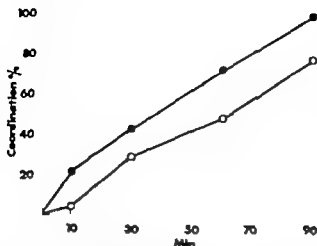


Fig. 1 The coordination of contractions in the cell groups over time at 10°C (O—O) and at 15°C (●—●). At every incubation time (abscissa) the percentage of coordinated cell groups (ordinate) is calculated from 19–35 cell groups. After 90 min of incubation the coordination at different temperatures differs significantly ($P < 0.05$).

earlier observed that myocardial cell from young animals functioned more regularly in these experimental conditions (Pelkonen & Tirri 1972). The rats were killed by decapitation and the hearts were removed and washed in ice-cold homogenization medium (HM) made according to Bloom (1970). It contained (mmoles/l) KCL 104, MgCl₂ 5, Na₂ATP 5, potassium oxalate 2, glucose 7 and potassium-phosphate buffer 18 at pH 7.0. The ventricles were dissected free from atria and homogenized in a cutter homogenizer (50 000 rpm) for 6–10 s in 10 ml of ice-cold HM. The homogenization did not simply break the cells but rather cut and tore the ventricles into cell groups of different sizes. Most of them were flat and elongated. The fragments selected for the experiments were 100–250 μ m in length being 6–8 cells broad, 3–4 cells long and one or two cells thick.

Coordination experiment

A Method using a video-tape recorder. The homogenate was kept on ice for 15 min to get the cell groups in bottom. A sample of 0.1 ml from the bottom was pipetted into a test tube within 0.7 ml of fresh HM or HM with the drug to be tested. Then a sample of 4–10 cell groups was transferred from this tube onto a depression slide and covered with a glass cover. The incubation times, the temperatures and the drug concentrations are given in the figures. The samples were promptly checked with a microscope with phase contrast optics and ocular micro-scale and the movements of each contracting cell group found were recorded on tape for 70–30 s with a TV camera and video-tape recorder. Later it was determined from the videotape whether the cell group was coordinated or not.

B Methods using photoamplifier and chart-recorder. In support of visual observation equipment was also used by which contractions of cell group could be registered. A sensitive photoamplifier was connected to the examination chamber (0.15 ml made on a slide). The cell group in

HM was placed on the bottom of the chamber between the tips of two glassfibres (\varnothing 0.05 mm) on a straight line. A cold light beam through one fibre passed the cleft with the cell group and was transmitted to a sensitive photoamplifier through another fibre. Contractions in the cell group changed the intensity of the light. The signal from the photoamplifier was displayed on a chart-recorder. The cell group was placed between the fibre tips with a thin glass capillary either by hand or using a micromanipulator. After 4–7 min caffeine was added with a micropipette (5 μ l). The test temperature was 10°C (room temperature).

Contraction velocity

After incubating the cell groups in a test tube for 30 min at 15°C the test tube was warmed to 20°C and a sample of cell groups was placed in a depression slide as described above. The microscope heating stage was adjusted to the testing temperature (20°C, 30°C or 35°C). A coordinated cell group was searched for and its contractions were recorded on a videotape for about 1 min. The contraction velocity was measured on a TV screen from a typical contraction wave in each cell group with the aid of a ocular scale and a stop-watch. The time intervals for measurement were about two seconds.

Statistics

The difference in coordination vs. uncoordination was tested with the 2×2 contingency table test and the coordination velocity difference was tested with the t -test. The limit of significance was chosen as 0.05.

RESULTS

Effects of time and temperature on the coordination

As stated previously (Pelkonen & Tirri 1972) single cells of a cell group usually contract in an uncoordinated way i.e. each muscle cell according to its own rhythm. In some cases however the action of the cell group becomes coordinated so that one cell served as a pacemaker starting the waves of contractions. We now observed that sometimes there may be two or more pacemaker cells in the group functioning alternatively. We also found that these uncoordinated contractions become more regular with time when the cell groups are incubated in HM (Fig. 1). The figure further shows that this spontaneous coordination of contractions is temperature-dependent. Immediately after the preparation of the cell group in the ice-cold HM all the cell groups functioned in an uncoordinated way. The number of the coordinated cell groups increased almost linearly with time of incubation so that after 90 min at 15°C all the cell groups studied were functioning in a coordinated way. However only 75% of the cell groups were coordinated when the

we observe any clear contracture in our experiments using azide concentrations up to 40 mM. The inhibition of spontaneous coordination by azide could be best explained by findings that azide inhibits Ca^{2+} release from the sarcoplasmic reticulum (Eaton *et al.* 1973).

If we assume that the Ca^{2+} -induced Ca^{2+} release initiates the contraction, the question arises whether the concentration of Ca^{2+} in HMI is sufficient to induce this. It is observed that only $\sim 4 \mu\text{M}$ concentration of outside Ca^{2+} is needed for the intracellular stimulation of Ca^{2+} release from microsomes (Kirchberger & Wong 1978). In our experiments Ca^{2+} concentration in the HMI was not determined, but there are determinations from similar media indicating Ca^{2+} concentrations of about 8–9 μM (Bloom & Mulholland 1976; Bloom 1978). At a steady state of Ca^{2+} influx and efflux it was estimated to be about 1 mM (Bloom 1978), which is still far above the level of 10^{-6} M that has been found to cause a cyclical Ca^{2+} release and uptake in isolated cardiac cells (Fabiato & Fabiato 1975a).

A possible sequence of events may be postulated: the rhythmic contractions which spontaneously become coordinated. At first the beating could be totally dependent on extracellular coordinated Ca^{2+} . This activates the myofilaments and participation of Ca^{2+} from the sarcoplasmic reticulum. The contractions are uncoordinated. A possible coordinated Ca^{2+} release from the sarcoplasmic reticulum would be masked by this extracellular Ca^{2+} . Later when Ca^{2+} uptake has proceeded, the role of the sarcoplasmic reticulum Ca^{2+} as a contraction inducer is increased. This Ca^{2+} is then able to diffuse to other cells initiating the coordinated type of contractions in a cell group. This would occur as a Ca^{2+} -induced Ca^{2+} release which is known to function cyclically (Fabiato 1975a). The presence of extracellular Ca^{2+} is, however, needed for the repeated activation of the Ca^{2+} release.

The work was supported by a grant from the National Research Council for Sciences.

REFERENCES

- BLOOM S. 1970 Spontaneous rhythmic contraction of separated heart muscle cells. *Science* 167 1727–1729.
- BLOOM S. 1971 Requirement for spontaneous contractility in isolated adult mammalian heart muscle cells. *Exptl Cell Res* 69: 17–24.
- BLOOM S. 1978 The relationship between calcium flux and beating in hyperpermeable heart muscle cells. *Biochim Biophys Acta* 514 206–212.
- BLOOM S., BRADY A. J. & LANGER, D. A. 1974 Calcium metabolism and active tension in mechanically disaggregated heart muscle. *J Mol Cell Cardiol* 6: 137–147.
- BLOOM S. & MULHOLLAND M. 1976 Calcium uptake by mechanically disaggregated heart muscle. *J Mol Cell Cardiol* 8 559–574.
- ENDO M. 1975 Condition required for calcium-induced release of calcium from the sarcoplasmic reticulum. *Proc Japan Acad* 51 467–472.
- ENDO M. 1977 Calcium release from the sarcoplasmic reticulum. *Physiol Rev* 57 71–108.
- ENTMAN M. L., BORNET E. P. & SCHWARTZ, A. 1977 Phasic components of calcium binding and release by canine cardiac r-relaxing system (sarcoplasmic reticulum fragments). *J Mol Cell Cardiol* 4 155–169.
- FABIATO A. & FABATO F. 1975 Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. *J Physiol* 49: 469–495.
- FABIATO A. & FABATO F. 1975b Effects of magnesium on contractile activation of skinned cardiac cells. *J Physiol* 249 497–517.
- FABIATO A. & FABATO F. 1977 Calcium release from the sarcoplasmic reticulum. *Circ Res* 40 119–179.
- HANBURG M. & GERGELY J. 1965 Studies on adenosine triphosphate supported calcium accumulation by cardiac subcellular particles. *J Biol Chem* 240: 2721–2728.
- KIRCHBERGER, M. A. & WONG D. 1978 Calcium efflux from isolated sarcoplasmic reticulum. *J Biol Chem* 253 6941–6945.
- LANGER, D. A. 1976 Events at the cardiac sarcolemma: localization and movement of contractile-dependent calcium. *Federation Proc* 35 1274–1278.
- PELKONEN M. & TIRRI R. 1972 The adrenergically mediated coordination of contraction in isolated cell groups from rat ventricular myocardium. *Experientia* 28: 1040–1041.
- THORENS, S. & ENDO M. 1975 Calcium induced calcium release and depolarization induced calcium release: their physiological significance. *Proc Japan Acad* 51 473–478.

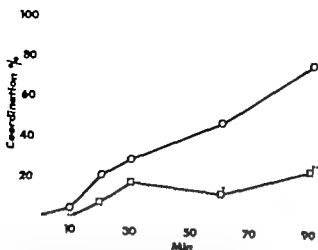


Fig. 4. The effect of azide on the coordination of the cell groups at 10°C. At every incubation time (abscissa) the percentage of coordinated cell groups (ordinate) is calculated. In the azide experiments 1–3 cell groups at every incubation time (□—□) and in the controls 3–35 cell groups at every incubation time (○—○) were used. The azide concentration was 5×10^{-4} M. The points marked with asterisk differ significantly from the controls ($P < 0.01$).

Velocity of the contraction wave

The velocity of the contraction wave was measured at 3 temperatures: at 20°C, at 30°C and at 35°C. It was surprising that the elevation of temperature had no effect at all on the velocities of the contraction waves. The results obtained were $90 \pm 7 \mu\text{m/s}$ ($n=12$), $100 \pm 6 \mu\text{m/s}$ ($n=11$) and $100 \pm 7 \mu\text{m/s}$ ($n=3$) respectively.

DISCUSSION

The results show that the contractions in the myocardial cell group become coordinated in 1–2 h after the disaggregation, so that usually one cell serves as a pacemaker. This coordination occurs spontaneously when incubated in HM and it is dependent on temperature.

Previously Pelkonen & Tirri (1972) suggested that the coordination wave was transferred electrically from cell to cell. According to current thinking this is implausible: no electrical activity can be observed in this ionic medium, since the cells are depolarized; moreover they can not be paced by a field stimulation (Bloom et al. 1974). According to these and our own results the most obvious mediator of the contraction wave seems to be Ca^{2+} . Its release from the sarcoplasmic reticulum and binding to the troponin C are the final steps in the

excitation-contraction coupling. When HM containing intracellular fluid was used the induction of contractions starts at a later stage, by-passing the sarcolemmal electrical stages. This idea explains why broken cells, e.g. a half of a cell contracted well as those with an intact sarcolemma.

A very slow speed (90–100 $\mu\text{m/s}$) of the contraction wave also indicates a nonelectrical type of events. It has been observed that in a myocardial cell group with a disrupted sarcolemma the contraction wave initiated by the calcium-induced calcium release propagated at 80–100 $\mu\text{m/s}$ (Fabiato & Fabiato 1975b). This similarity may confirm that Ca^{2+} plays a conclusive role in propagating the contraction wave in a cell but probably also between the cells in these circumstances. In the latter case this assumes that Ca^{2+} can quite freely diffuse through cell membranes, especially through the intercalated discs. Actually in some cases it seems that the contraction propagated transversely through the ordinary sarcolemma from cell to cell.

The spontaneous coordinations of contractions are clearly temperature-dependent: an elevation of the temperature hastens the coordination. Bloom (1970) observed that elevation of the temperature under the same conditions as we used has a positive chronotropic action on myocardial cells. He also found that Ca^{2+} uptake was dependent of ATP and the temperature (Bloom & Mulholland 1976). The enzymatic mechanisms connected to Ca^{2+} uptake and release: influx and efflux may in fact be involved in the coordination of contractions. It is surprising that the temperature had almost no effect at all on the speed of the contraction wave thus indicating a non-enzymatical event, e.g. diffusion of Ca^{2+} in the propagation of the wave.

It has been observed that caffeine increases the calcium induced calcium release when the sarcoplasmic reticulum is loaded over a certain level of calcium (Endo 1975). This could explain a stimulating effect of caffeine on the coordination. The steady contracture followed when Ca^{2+} uptake was not able to compensate an enhanced release due to greater concentration of caffeine in HM. Azide has a depressing effect on Ca^{2+} uptake into the mitochondria (Famburg & Gergely 1965). There are also observations that azide can cause contracture of the skinned heart cells (Fabiato & Fabiato 1975a) and an increase of resting tension in heart fragments (Bloom et al. 1974). These effects suggest a greater free Ca^{2+} level inside the cells. We could

we observe any clear contracture in our experiments using azide concentrations up to 50 mM. The inhibition of spontaneous coordination by azide could be best explained by findings that azide inhibits Ca^{2+} release from the sarcoplasmic reticulum (Eaton et al. 1973).

If we assume that the Ca^{2+} -induced Ca^{2+} release stimulates the contraction, the question arises whether the concentration of Ca^{2+} in HM is sufficient to induce this. It is observed that only $\sim 4 \mu\text{M}$ concentration of outside Ca^{2+} is needed for the mechanical stimulation of Ca^{2+} release from microsomes (Kirschberger & Wong 1978). In our experiments Ca^{2+} concentration in the HM was not determined, but there are determinations from similar systems indicating Ca^{2+} concentrations of about 8–9 μM (Bloom & Mulholland 1976; Bloom 1978). At a critical state of Ca^{2+} influx and efflux it was estimated to be about 1 nM (Bloom 1978) which is still higher than the level of 10^{-7} M that has been shown to cause a cyclical Ca^{2+} release and uptake in skinned cardiac cells (Fabiato & Fabiato 1975 a).

A possible sequence of events may be postulated concerning the rhythmic contractions which spontaneously become coordinated. At first the beating could be totally dependent on extracellular Ca^{2+} concentration. This activates the myofilaments without participation of Ca^{2+} from the sarcoplasmic reticulum. The contractions are uncoordinated. A possible coordinated Ca^{2+} release from the sarcoplasmic reticulum would be masked by this extracellular Ca^{2+} . Later when Ca^{2+} uptake has proceeded, the role of the sarcoplasmic reticulum Ca^{2+} as a contraction inducer is increased. Thus Ca^{2+} is then able to diffuse to other cells initiating the coordinated type of contractions in a cell group. This would occur as a Ca^{2+} -induced Ca^{2+} release which is known to function cyclically (Fabiato & Fabiato 1975 a). The presence of extracellular Ca^{2+} is, however, needed for the repeated activation of the Ca^{2+} release.

This work was supported by a grant from the National Research Council for Sciences.

REFERENCES

- BLOOM S. 1970. Spontaneous rhythmic contraction of separated heart muscle cells. *Science* 167 1777–1779.
- BLOOM S. 1971. Requirements for spontaneous contractility in isolated adult mammalian heart muscle cells. *Exptl Cell Res* 69 17–31.
- BLOOM S. 1978. The relationship between calcium flux and beating in hyperpermeable heart muscle cells. *Biochim Biophys Acta* 514 706–712.
- BLOOM S., BRADY A. J. & LANGER G. A. 1974. Calcium metabolism and active tension in mechanically disaggregated heart muscle. *J Mol Cell Cardiol* 6 137–147.
- BLOOM S. & MULHOLLAND M. 1976. Calcium uptake by mechanically disaggregated heart muscle. *J Mol Cell Cardiol* 8 559–574.
- ENDO M. 1975. Condition required for calcium-induced release of calcium from the sarcoplasmic reticulum. *Proc Japan Acad* 51 467–472.
- ENDO M. 1977. Calcium release from the sarcoplasmic reticulum. *Physiol Rev* 57 71–108.
- ENTMAN M. L., BORNET E. P. & SCHWARTZ, A. 1972. Physic components of calcium binding and release by cardiac cardiac relaxing system (sarcoplasmic reticulum fragments). *J Mol Cell Cardiol* 4 155–169.
- FABIATO A. & FABATO F. 1975. Contractions induced by calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. *J Physiol* 49 469–495.
- FABIATO A. & FABATO F. 1975 b. Effects of magnesium on contractile activation of skinned cardiac cells. *J Physiol* 49 497–517.
- FABIATO A. & FABATO F. 1977. Calcium release from the sarcoplasmic reticulum. *Circ Res* 40 119–129.
- FANBURG, B. & GERGELY J. 1965. Studies on adenosine triphosphate supported calcium accumulation by cardiac subcellular particles. *J Biol Chem* 240 2771–2778.
- KIRSCHBERGER M. A. & WONG H. 1978. Calcium efflux from isolated sarcoplasmic reticulum. *J Biol Chem* 253 6941–6945.
- LANGER, G. A. 1976. Events in the cardiac sarcolemma: localization and movement of contractile-dependent calcium. *Federation Proc* 35 1274–1278.
- PELKONEN K. & TIRRI R. 1977. The adrenergically mediated coordination of contraction in isolated cell groups from rat ventricular myocardium. *Experientia* 28, 1040–1041.
- THORENS, S. & ENDO M. 1975. Calcium induced calcium release and depolarization-induced calcium release: their physiological significance. *Proc Japan Acad* 51 473–478.

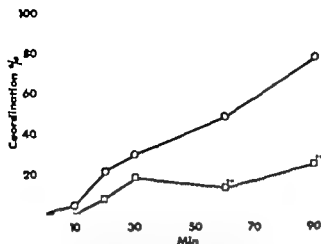


Fig. 4. The effect of azide on the coordination of the cell groups at 10°C. At every incubation time (abscissa) the percentage of coordinated cell groups (ordinate) is calculated. In the azide experiments 1-3 cell groups at every incubation time (□-□) and in the controls 3-35 cell groups at every incubation time (○-○) were used. The azide concentration was 5×10^{-4} M. The points marked with asterisks differ significantly from the controls ($P < 0.01$).

Velocity of the contraction wave

The velocity of the contraction wave was measured at 3 temperatures: at 20°C, at 30°C and at 35°C. It was surprising that the elevation of temperature had no effect at all on the velocities of the contraction waves. The results obtained were $90 \pm 7 \mu\text{m/s}$ ($n=17$), $100 \pm 6 \mu\text{m/s}$ ($n=11$) and $100 \pm 7 \mu\text{m/s}$ ($n=3$) respectively.

DISCUSSION

The results show that the contractions in the myocardial cell group become coordinated in 1-2 h after the disaggregation, so that usually one cell serves as a pacemaker. This coordination occurs spontaneously when incubated in HM and it is dependent on temperature.

Previously Pelkonen & Tiiri (1972) suggested that the coordination wave was transferred electrically from cell to cell. According to current thinking this is implausible: no electrical activity can be observed in this ionic medium, since the cells are depolarized; moreover they can not be paced by a field stimulation (Bloom et al. 1974). According to these and our own results the most obvious mediator of the contraction wave seems to be Ca^{2+} . Its release from the sarcoplasmic reticulum and binding to the troponin C are the final steps in the

excitation-contraction coupling. When HM resembling intracellular fluid was used the induction of contractions starts at a later stage, by-passing the sarcolemmal electrical stages. This idea explains why broken cells, e.g. a half of a cell, contracted as well as those with an intact sarcolemma.

A very slow speed (90-100 $\mu\text{m/s}$) of the contraction wave also indicates a nonelectrical type of events. It has been observed that in a myocardial cell group with a disrupted sarcolemma, the contraction wave initiated by the calcium-induced calcium release propagated at 80-100 $\mu\text{m/s}$ (Fabiato & Fabiato 1975b). This similarity may confirm that Ca^{2+} plays a conclusive role in propagating the contraction wave in a cell but probably also between the cells in these circumstances. In the latter case this assumes that Ca^{2+} can quite freely diffuse through cell membranes, especially through the intercalar discs. Actually in some cases it seems that the contraction propagated transversely through the ordinary sarcolemma from cell to cell.

The spontaneous coordinations of contraction are clearly temperature-dependent: an elevation of the temperature hastens the coordination. Bloom (1970) observed that elevation of the temperature in the same conditions as we used has a positive chronotropic action on myocardial cells. He also found that Ca^{2+} uptake was dependent of ATP and the temperature (Bloom & Mulholland 1976). The enzymatic mechanisms connected to Ca^{2+} uptake and release, influx and efflux may in fact be involved in the coordination of contractions. It is surprising that the temperature had almost no effect at all on the speed of the contraction wave thus indicating a non-enzymatical event, e.g. diffusion of Ca^{2+} in the propagation of the wave.

It has been observed that caffeine increases the calcium-induced calcium release when the sarcoplasmic reticulum is loaded over a certain level of calcium (Endo 1975). This could explain a stimulating effect of caffeine on the coordination. The steady contraction followed when Ca^{2+} uptake was not able to compensate an enhanced release due to greater concentration of caffeine in HM. Azide has a depressing effect on Ca^{2+} uptake into the mitochondria (Famburg & Gergely 1965). There are also observations that azide can cause contracture of the skinned heart cells (Fabiato & Fabiato 1975a) and an increase of resting tension in heart fragments (Bloom et al. 1974). These effects suggest a greater free Ca^{2+} level inside the cells. We could

Effect of intraluminal pH on the release of somatostatin and gastrin into antral bulbar and ileal pouches of conscious dogs

KERSTIN UYVÄS-WALLENSTEN SUAD EFENDIC CATIA JOHANSSON
LARS SJÖDIN and PETER D. CRANWELL

Department of Pharmacology at Karolinska Institute, Department of Endocrinology at Karolinska Hospital
GI Clin Department of Medicine at Karolinska Hospital, Stockholm, Department of Drug Control at
National Board of Health and Welfare, Uppsala and School of Agriculture, La Trobe University, Australia

UYVÄS-WALLENSTEN K, EFENDIC S, JOHANSSON C, SJÖDIN L & CRANWELL P. Effect of intraluminal pH on the release of somatostatin and gastrin into antral, bulbar and ileal pouches of conscious dogs. *Acta Physiol Scand* 1980, 110: 391-400. Received 11 March 1980 ISSN 0014-6772. Department of Pharmacology Karolinska Institute, Department of Endocrinology and Medicine Karolinska Hospital Stockholm, Sweden, Department of Drug Control, National Board of Health and Welfare Uppsala, Sweden and School of Agriculture, La Trobe University Australia.

Experiments were performed on conscious dogs with chronic pouches of the antrum, the duodenal bulb or the ileum, which were perfused with solutions of varying pH. Gastrin and somatostatin levels were measured in the perfusates. When the pouches were perfused with 0.15 M NaCl only small amounts of gastrin and somatostatin (1 pmol/min) were released into the lumen of the antrum and of the duodenal bulb. By lowering pH of the perfusion fluid pH dependent release of somatostatin was induced into the lumen of the antrum and the duodenal bulb. Perfusion with 0.1 M HCl caused large output of somatostatin (6-60 pmol/min) into the pouches. The upper pH limit for stimulation of the intraluminal or intrabulbar somatostatin release appeared to be ~pH 3-4. Somatostatin was also released into ileal perfusates at intraluminal pH below 3-4. Lowering of pH in the antral pouches caused an increased intraluminal gastrin release, which was quantitatively less expressive than that of somatostatin. Occasionally also the gastrin release into the duodenal bulb increased during perfusion with 0.1 M HCl, whereas no such release was induced by acidification of the lumen of the ileum. It is suggested that the inhibition of gastrin release observed at low intraluminal pH is mediated by a local effect of somatostatin, since this peptide is released in a pH dependent manner in the antropyloric region. It is also suggested that acidification of any region of the gastro-intestinal tract will stimulate the release of peptides from all endocrine cells of the open type, probably by an unspecific effect on the membrane. Thus both gastrin and somatostatin are released by acidification of the antrum, but in the presence of high local levels of somatostatin, the release of gastrin is substantially inhibited.

Key words: Somatostatin, gastrin, release, inhibition, antral pH, intraluminal secretion.

The antral mucosa is the main producer of the gastric acid stimulating hormone gastrin. Recently somatostatin, shown to inhibit gastric acid secretion, has been demonstrated to inhibit gastrin release and by acting directly on the parietal cells (Bloom et al. 1974; Le Razi et al. 1975; Raptin et al. 1975; Uväs-Wallensten, Efendic & Luft 1977) has been demonstrated to inhibit endocrine cells in the mucosa of the gastro-intestinal tract. The antropyloric region is particularly rich in somatostatin containing D-cells, some of

which are located close to the gastrin containing G-cells (Rufener et al. 1975).

Acidification of the antrum and the proximal duodenum is known to inhibit gastric acid secretion. This effect is claimed to be in part mediated via a diminished output of gastrin (Andersson & Olbe 1963; Nilsson et al. 1972; Csendes, Wajsb & Grossman 1972; Wajsb, Richardsson & Fordtran 1975) and in part via release of inhibitory hormones, which block acid secretion at the

Table 1a and b pH in the pouch perfusates from the antrum (a) and the duodenal bulb (b)

The pouches are perfused with 0.15 M NaCl during the first (0-120 min) and last (180-300 min) 2 h periods, i.e. samples 1-4 and 7-10. During the middle hours (no statistics) the pouches were perfused with 0.1 M HCl (pH ~1.2) or citrate-phosphate buffer pH ~3, 4 or 5. Note the lag in changes of perfusate pH back to basic pH is decreased and increased.

Table 1

No. of sample	Collection time (min)	0.1 M HCl pH	pH			
			pH 1	pH 3	pH 4	pH 5
1	0	6.5	6.5	6.1	5.8	6.1
2	30	6	6.7	6.0	6	5.9
3	60	6.3	6.7	6.0	4.8	5.6
4	90	6.3	6.7	5.9	5.7	5.6
5	120	4.5	4.5	5.3	5.0	5.1
		1	2.5	3	4.0	5.1
6	150	1.4	2.4	3	4.0	5.1
		1.3	2.3	3	4.0	5.1
7	180	1.5	4	3	4.1	5.1
		1.5				
8	210	8	4	3.1	4.1	5
		2.8				
9	240	4.4	4	3.6	4.3	5.3
		4.7				
10	270	4.7	5.4	4.5	5	5.7
		6.0				
	300					

Table 1b

No. of sample	Collection time (min)	0.1 M HCl pH	pH			
			pH 2	pH 3	pH 3	pH 5
1	0	7.4	7.2	6.9	7.2	6.8
2	30	7	7.3	7.0	7.3	6.8
3	60	7.1	7.3	7.3	7.2	6.9
4	90	7.1	7.3	7.3	7.2	7.0
5	120	2.6	4.3	3.8	2.7	6.8
		1.6	2.6	3.1	5.1	5.8
6	150	1	2.4	3.1	4.0	5.1
		1.3	2.4	3.1	4.0	5.1
7	180	1.5	2.6	3.2	4.1	5.1
		1.6				
8	210	3.0	3.4	3.5	4.6	6
9	240	5.6	4.9	4.9	5.7	6.2
10	270	6.6	6.1	6.7	6.6	6.9
	300					

Whereas pH of bulbar perfusates averaged ~7. The maximal decrease in pH was not obtained in antral or bulbar perfusates until ~70 min after starting the perfusion with the solutions of lower pH. A similar delay until neutral pH was reached again, was ob-

served during the reperfusion with 0.15 M NaCl. The lowest pH recorded during perfusion with 0.1 M HCl was ~pH 1. in both antral and bulbar perfusates (Fig. 1 and c).

The mean values \pm S.E. of the gastrin and somatostatin release per 30 min period at the different pH levels are depicted in Table 2a and b. The values are based on the mean release obtained in each individual dog.

Ant. perfusates

Three dogs with chronic antral pouches were used in this series. During the control period when the antrum was perfused with 0.15 M NaCl on the average 0.9 pmol/min of gastrin and 0.4 pmol/min of somatostatin were released into the lumen, i.e. the ratio somatostatin/gastrin being 0.5/1. (These values are based on all control values $n=3$...) (Table 2a).

After the control period either 0.1 M HCl ($n=13$) citrate-phosphate-buffer pH ~ (= $n=18$) pH 3 ($n=12$) pH 4 ($n=4$) or pH 5 ($n=10$) was introduced into the pouches. When perfusate pH was decreased an increase in somatostatin values was observed. The lower the pH the more somatostatin appeared in the perfusates, during perfusion with 0.1 M HCl as much as 50 pmol/min was released. A small release of somatostatin was observed even at pH 4 (Table 2a Fig. 1).

A slight increase in gastrin output was also observed at the lowest pHs (Fig. 1a Table 2a). However on a molar base the ratio somatostatin/gastrin was ~5/1 in these experiments (Fig. 1). When 0.15 M NaCl was reintroduced into the perfusion system somatostatin and gastrin values returned towards basal levels (Fig. 1a b and c).

Bulbar perfusates

Three dogs with bulbar pouches were used in this series. The number of experiments performed at the different pHs were 0.1 M HCl ($n=8$) pH 2.2 ($n=10$) pH 3 ($n=3$) pH 4 ($n=$) and pH 5 ($n=2$). Only very low amounts of somatostatin could be detected in perfusates of the duodenal bulb during perfusion with 0.15 M NaCl (~0.1 pmol/min) (Table 2b). When the pH was lowered however a substantial increase of intraluminal release of somatostatin occurred. As in the antrum, perfusion with acid caused a large output of somatostatin (~20 pmol/min). The upper threshold for the stimulation

parietal cell level (Thompson 1966 Wheeler 1974 Uvnäs 1971). Since somatostatin having both these properties is present in high amounts in the antropyloric mucosa it was tempting to investigate the possibility that the inhibitory effect on gastrin release and gastric acid secretion induced by low antral pH might be mediated through a release of endogenous somatostatin. In support of this theory are the findings that D-cells degranulate during perfusion of the antropyloric lumen with acid (Fujita & Kobayashi 1971) and those demonstrating that somatostatin is released into the antral lumen of anesthetized cats in response to electrical vagal stimulation only during perfusion of the antrum with 0.1 M HCl whereas no such release occurs at neutral intraluminal pH (Uvnäs Wallensten Efendic & Luft 1977). Furthermore portal vein somatostatin levels increase in anesthetized dogs during instillation of acid into the stomach (Gustavsson & Lundqvist 1978 Shusdzharo et al 1978).

The aim of the present series of experiments was to investigate whether the inhibitory effect on gastrin release induced by acidification of the antrum is mediated by somatostatin. Therefore we wanted to study the influence of pH in the antropyloric lumen on the release of gastrin and somatostatin. For this purpose dogs were provided with chronic pouches of the antrum or of the duodenal bulb and these were perfused with solutions of varying pH.

Somatostatin levels in plasma are difficult to measure and represent a mixture of somatostatin originating from several sources. However gastrointestinal somatostatin is like gastrin released into the lumen of the antral and duodenal bulb (probably reflecting a bidirectional release of the peptide into the lumen and into the circulation) (Uvnäs Wallensten & Rehfeld 1976 Uvnäs Wallensten Efendic & Luft 1977 1978 Uvnäs Wallensten 1977a 1978a Uvnäs Wallensten & Efendic 1978). To circumvent these two problems we have chosen to study the release of gastrin and somatostatin by analyzing antral or bulbar perfusates for their content of these peptides.

METHODS

Surgical procedures
Seven mongrel dogs weighing 16–1 kg were provided with various gastrointestinal pouches. The dogs were allowed a period of 3 weeks for recovery after each operation.

Series A In 3 dogs vagally innervated gastric fundic pouches (Pavlov type) were constructed by a slight modification of the method of Thomas (1941). At a second operation an antral pouch was isolated from the main stomach by the construction of a double mucosal wall after the antrum corpus border was visualized by the pH paper indicator technique (Andersson 1960, Öhr 1961). Gastrointestinal continuity was restored by gastric junostomy.

Series B Four dogs were equipped with vagally innervated fundic pouches (Pavlov type). In a second operation a double mucosal wall was constructed in the pylorus in 3 of the dogs and gastrointestinal continuity was restored by gastric junostomy. At a third operation the duodenum was transected just proximal to the entrance of the common bile duct. The isolated bulb was anastomosed to a resected loop of the terminal ileum which was brought out to the abdominal wall in a cutaneous fistula.

In the fourth dog, no pouch of the bulb was constructed. A 10 cm long loop of the terminal ileum was isolated and one end of the loop was brought to the abdominal wall as a fistula.

Each experiment was performed after an 18 h fasting period. Before the experiments a double lumen catheter was introduced into the pouch in order to allow perfusion. Both antral bulbar and ileal pouches were perfused for 5 h with solutions at room temperature at a rate of 1–2 ml/min and at a very slight overpressure (10 cm H₂O). During the first and last 1 h the pouches were perfused with 0.15 M NaCl. During the mid third hour NaCl was exchanged for 0.1 M HCl or buffer solutions of pH 2, 3, 4 or 5 (MacIlvaine's standard buffer containing 0.1 M citric acid and 0.1 M NaH₂PO₄). 3–5 expts. were performed at each pH on each dog except at pH 3, 4 and 5 in the dogs with bulbar pouches. Due to the very small effects observed only a total of 3 expts. were performed at these pHs. On the dog with the ileal pouch only 1 expt. at pH 2, 3 and 4 respectively were performed.

Treatment of sample

Samples of the pouch perfusate were collected in 15 or 30 min portions. Immediately after collection, the pH of the perfusates was measured and adjusted to 7 by addition of 0.1 M NaOH using phenolphthalein as indicator. The samples were heated to 100° for 1 min and were then kept frozen (–70°) until assayed for gastrin and somatostatin.

Gastrin and somatostatin levels were measured with radioimmunoassays as described by Nilsson (1975) and Efendic et al (1978) respectively.

The release rate of the peptides (pmol/min) is calculated by multiplying the concentration of gastrin and somatostatin in the perfusates (pmol/ml) with the flow rate of the perfusate (ml/min).

RESULTS

pH of the pouch perfusates is depicted in Table 1a and b. When the antral pouches were perfused with 0.15 M NaCl pH of the perfusates was ~6.

Table 2a and b. Influence of pH on the release of gastrin (G) and somatostatin (S) (pmol/min \pm S.E.) in pouch perfusates of the antrum (a) or the duodenal bulb (b)

The pouches were perfused with 0.15 M NaCl during the first (0-120 min) and last (180-300 min) 2 h period. In samples 1, 14 and 14b. During the midboxer the pouches were perfused with 0.1 M HCl (pH \sim 1.2) or citrate phosphate buffer pH 3.4 or 5 (in italics).

Table 2a

No of sample	Collection time (min)		pH 1.2	pH 2.2	pH 3	pH 4	pH 5
1	0	G	893 \pm 237	649 \pm 85	817 \pm 51	827 \pm 194	406 \pm 115
		S	294 \pm 75	435 \pm 163	288 \pm 122	675 \pm 158	325 \pm 763
2	30	G	875 \pm 147	877 \pm 236	1 077 \pm 15	712 \pm 40	1 03 \pm 40
		S	26 \pm 79	451 \pm 110	348 \pm 193	799 \pm 102	996 \pm 268
3	60	G	1 229 \pm 46	1 029 \pm 4	1 004 \pm 138	643 \pm 251	869 \pm 307
		S	180 \pm 47	498 \pm 221	470 \pm 701	307 \pm 197	794 \pm 223
4	90	G	940 \pm 772	946 \pm 106	917 \pm 797	541 \pm 81	683 \pm 13
		S	305 \pm 92	359 \pm 171	649 \pm 310	155 \pm 75	176 \pm 99
5	120	G	1 976 \pm 548	1 025 \pm 333	694 \pm 234	753 \pm 112	565 \pm 190
		S	5 490 \pm 643	1 399 \pm 318	849 \pm 307	615 \pm 453	215 \pm 141
6	150	G	7 810 \pm 618	1 000 \pm 252	450 \pm 19	487 \pm 153	528 \pm 45
		S	36 \pm 49 10 787	5 654 \pm 374	2 783 \pm 1 715	1 008 \pm 868	2 \pm 76
7	180	G	9 577 \pm 2 752	1 097 \pm 195	510 \pm 87	307 \pm 120	418 \pm 31
		S	52 984 \pm 23 890	6 702 \pm 3 675	354 \pm 1 477	677 \pm 457	177 \pm 72
8	210	G	4 375 \pm 484	845 \pm 93	374 \pm 82	379 \pm 140	371 \pm 81
		S	6 867 \pm 1 831	5 471 \pm 3 804	1 279 \pm 987	377 \pm 186	143 \pm 82
9	240	G	2 106 \pm 149	653 \pm 151	340 \pm 84	657 \pm 81	422 \pm 136
		S	2 452 \pm 645	1 367 \pm 371	1 073 \pm 932	282 \pm 164	147 \pm 100
10	270	G	2 253 \pm 224	989 \pm 90	567 \pm 188	424 \pm 152	384 \pm 163
		S	1 592 \pm 351	906 \pm 179	542 \pm 456	159 \pm 73	191 \pm 168

Table 2b

No of sample	Collection time (min)		pH 1.2 =	pH 2.2 =10	pH 3 =8	pH 4 =	pH 5 =2
1	0	G	217 \pm 110	88 \pm 78	18 \pm 9.3	34	5
		S	103 \pm 7	63 \pm 25	112 \pm 59	60	52
2	30	G	323 \pm 235	56 \pm 49	26 \pm 13	0	7
		S	124 \pm 71	61 \pm 19	43 \pm 6	54	73
3	60	G	208 \pm 139	29 \pm 26	29 \pm 17	0	0
		S	54 \pm 14	58 \pm 3	31 \pm 12	19	88
4	90	G	365 \pm 80	11 \pm 10	29 \pm 17	0	11
		S	179 \pm 29	72 \pm 12	133 \pm 76	41	75
5	120	G	545 \pm 262	50 \pm 21	15 \pm 8	9	9
		S	2 670 \pm 1 309	840 \pm 229	10 \pm 7	112	114
6	150	G	1 723 \pm 361	32 \pm 7	91 \pm 74	11	0
		S	21 165 \pm 3 648	1 282 \pm 598	473 \pm 276	180	112
7	180	G	1 562 \pm 1 033	171 \pm 111	96 \pm 21	2	0
		S	17 564 \pm 3 128	1 795 \pm 1 233	421 \pm 143	230	86
8	210	G	993 \pm 705	132 \pm 72	12 \pm 9	20	0
		S	10 492 \pm 3 250	797 \pm 534	205 \pm 63	293	228
9	240	G	619 \pm 507	88 \pm 54	39 \pm 39	0	0
		S	1 842 \pm 465	122 \pm 39	112 \pm 13	181	130
10	270	G	551 \pm 390	33 \pm 12	33 \pm 19	7	0
		S	1 054	59 \pm 18	7 \pm 14	188	96

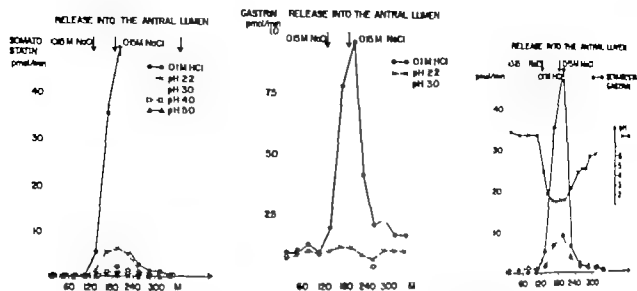


Fig. 1a Influence of pH on the release of somatostatin (pmol/min) into the antral lumen of conscious antral pouch dogs. The pouches were perfused with 0.15 M NaCl for a 1 h period at the beginning and end of each experiment. 1 h perfusion (between the arrows) with 0.1 M HCl or with buffer solutions of pH 2, 3, 4 and 5 was performed between the two control saline infusions.

Fig. 1b Influence of pH on the release of gastrin (pmol/min) into the antral lumen of conscious antral pouch dogs. The experiments were performed as described in the text of Fig. 1a.

Fig. 1c Influence of pH on the release of somatostatin and gastrin (pmol/min) during perfusion of the antral pouch with 0.1 M HCl. Note that the output of both gastrin and somatostatin peaks simultaneously, i.e. when the intraluminal pH reaches its minimum level.

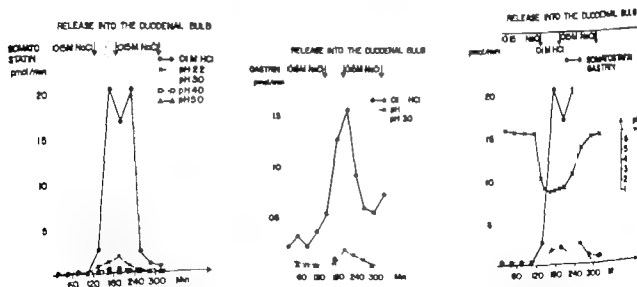


Fig. 2a Influence of pH on the release of somatostatin (pmol/min) into the bulbular lumen of conscious duodenal bulb dogs. The experiments were performed as described in Fig. 1a.

Fig. 2b Influence of pH on the release of gastrin (pmol/min) into the bulbular lumen of conscious duodenal bulb dogs. The experiments were performed as described in Fig. 1a.

Fig. 2c Influence of pH on the release of somatostatin and gastrin (pmol/min) on perfusion of the duodenal pouches with 0.1 M HCl. Note that the outputs of gastrin and somatostatin peak simultaneously, i.e. when the intrabulbar pH reaches its minimum level.

the immunoreactive material found in the antral and duodenal perforates really corresponds to gastrin and somatostatin respectively.

As to the second question posed above, the following observations indicate that acidification of the antropyloric region in fact causes a secretion of somatostatin and not just a release due to the damage of mucosal cells. The increased appearance of somatostatin (and gastrin) observed during perfusion with 0.1 M HCl ceased when the pH of the perfusion medium was restored to neutrality. This could not be the case if perfusion with HCl had induced an irreversible mucosal damage. Furthermore a significant output of somatostatin occurred into the antral and duodenal lumen also at pH 3-4 at which pH mucosal damage is not likely to occur. The reversed ratio between somatostatin and gastrin amounts in neutral and acid perfusates is difficult to reconcile with the damage theory.

Also supporting the idea that low intraluminal pH causes an active release of somatostatin and gastrin is the finding that peripheral plasma somatostatin and gastrin levels increase in parallel with the intraluminal output of the peptides (Uvnäs-Wallensten *et al.* to be published). Thus low pH within the antrum or duodenal bulb causes the same bidirectional peptide response just as do electrical vagal stimulations or injections of ACh, bombesin, apomorphine or sulphonic drugs (Uvnäs-Wallensten 1977; Uvnäs-Wallensten, Efendic & Luft 1971; Uvnäs-Wallensten *et al.* 1978; Uvnäs-Wallensten, Lundberg & Efendic 1978; Uvnäs-Wallensten, Blom & Johansson 1980).

What then is the physiological role of the pH dependent lateral release of somatostatin. In our view the hormones released into the gastrointestinal lumen may not necessarily exert any effects *per se*. The intraluminal secretion might be a spill over phenomenon reflecting high tissue levels of the peptides. The hormones might reach the lumen by intercellular diffusion, after having been released from the basal and lateral parts of the cells. For a more elaborate discussion on this matter see (Uvnäs-Wallensten (1977, 1980); Uvnäs-Wallensten, Efendic & Johansson (1980)).

Another interesting question is the possible physiological role of a pH controlled release of somatostatin within the antropyloric mucosa.

It is generally accepted that acidification of the antrum and proximal duodenum inhibits gastric acid

secretion in two ways: by reducing the release of antral gastrin (for references see below) and by stimulating the release of inhibitory humoral agents such as antral chalone (Thompson 1966; Wheeler 1974) and bulbogastrone (Uvna 1971). The inhibitory effect of acid antral pH on stimulated gastrin release is well documented. Thus in dogs less gastrin is released in response to shamfeeding or insulin hypoglycemia when antral pouches are perfused with acid than when they are perfused with 0.15 M NaCl (Nilsson *et al.* 1972; Csendes, Walsh & Grossman 1977), but only few attempts have been made to quantitatively correlate the gastrin release in response to a certain stimulus to various intragastric pHs. Howe *et al.* (1975) found that in humans no gastrin release was obtained by amino acid stimulation when pH of the gastric content was maintained at pH < 2.5 whereas release responses of the same magnitude were obtained at pH 3-4 and 5.5. More detailed pH studies have been performed in earlier experiments on dogs in which gastrin release was measured indirectly as the rate of HCl secretion. Thus Andersson & Olbe (1963) showed that the shamfeeding induced acid secretion from a Pavlov Pouch was not reduced at intraluminal pHs > 2.5. A slight inhibition occurred at pH 1-1.5 whereas a substantial suppression of the HCl response was induced at pH 1-1.5.

Infusions of somatostatin mimic the effect of low antral pH on the release of gastrin. Thus infusion of somatostatin inhibits the release of gastrin induced by shamfeeding and insulin hypoglycemia in man (Konturek *et al.* 1976; Schrupp *et al.* 1976; Raptin *et al.* 1975; Le Roith *et al.* 1975; Bloom *et al.* 1974) by feeding and by I.V. insulin and arginine in dogs (Barros, Bloom & Baron 1978; Ishida 1976) and by feeding and electrical vagal stimulation in cats (Albinus *et al.* 1975) and Uvnäs-Wallensten, Efendic & Luft (1977).

The results by Walsh, Richardson & Fordtran, and by Andersson & Olbe are in good agreement with the hypothesis that the inhibition of antral gastrin release observed at low intraluminal pH might be mediated by locally released somatostatin. Accordingly large amounts of somatostatin were released during perfusion of the antrum and the duodenal bulb with 0.1 M HCl and a profound inhibition of gastrin release and acid secretion was observed whereas a still significant but tenfold lower somatostatin release was observed at pH

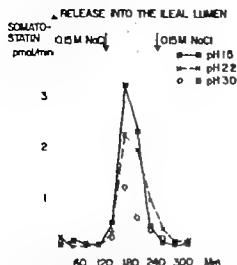


Fig. 2 Influence of pH on the release of somatostatin (pmol/min) into an ileal pouch during perfusion with solutions of pH 1.5 and 3.

of the somatostatin release was \sim pH 3–4 (Fig. 2a, Table 2b).

A 2–3 fold increase of the intraduodenal gastrin release was also observed during perfusion with 0.1 M HCl (Fig. 2b) the molar ratio somatostatin/gastrin being around 10 (Fig. 2c).

Ileal perfusates

Two perfusions at pH 1.5 and 3 respectively were performed in a dog with an ileal pouch. The perfusions were done exactly in the same way as in the dogs with antral and bulbar pouches. We found that lowering the pH of the perfusion medium (to 1.5, 2 and 3) caused a pH dependent increase of the intra ileal somatostatin release just as in the antral and in the bulbar pouches (Fig. 3). No gastrin release was observed into the ileal lumen either at neutral or at lower pHs.

DISCUSSION

In the present study we have shown that lowering of pH in antrum and duodenal bulb pouches causes a pH dependent output of somatostatin like immunoreactivity (SLI) into the lumen. During the perfusion with 0.1 M HCl on the average 50 and 70 pmol/min were released into the antrum and the bulb respectively. The upper pH threshold for activation of the somatostatin release appeared to be \sim pH 3–4. A small but significant increase of the SLI also occurred into ileal perfusates during perfusion of the ileal pouch with solutions of pH 1.5–3.

To our surprise the output of gastrin regular increased into antral and sometimes also into duodenal perfusates in response to intraluminal acidification. However, in comparison with the output of somatostatin the release of gastrin was small and only occurred on strong acidification, i.e. during perfusion of the antrum with 0.1 M HCl.

The occurrence of gastrin like immunoreactivity in antral perfusates of dogs is in agreement with studies performed by Andersson & Nilsson (1974) and by Rehfeld & Uvnäs Wallensten (1978). Furthermore both gastrin and somatostatin-like immunoreactivity has been shown to be released into the antral and duodenal lumen of cats in response to vagal stimulation and perfusion with alkaline solutions (Uvnäs Wallensten 1977, 1978, 1978a; Uvnäs Wallensten & Efendic 1978; Uvnäs Wallensten, Efendic & Luft 1977a, 1978; Uvnäs Wallensten et al 1979). Human gastric instillates have also been shown to contain gastrin and somatostatin-like immunoreactivity (Knight et al 1978; Wäsen et al. *in press*).

Some questions may be raised however: Do the immunoreactive materials observed in the acid perfusates really correspond to the two peptides in question and does acid pH increase the release of the peptides into the lumen by activating the inherent secretory mechanism? Or does acid pH just by damaging the mucosa lead to a high luminal content of gastrin and somatostatin deriving from exfoliated mucosal cells?

As to the first question. The gastrin like immunoreactivity appearing in gastric juice and neutral antral perfusates of the cat has been identified as gastrin 17 by means of gel filtration, whereas antral perfusates of dogs have been shown to contain both gastrin 17 and 34. The corresponding types of gastrin are released into the circulation of cats and dogs respectively (Uvnäs Wallensten & Rehfeld 1976; Rehfeld & Uvnäs Wallensten 1978). The somatostatin-like immunoreactivity has been characterized by Dr Pradayrol (Toulouse, France) by combining radioimmunological technique and high pressure liquid chromatography. He has been able to show that the SLI of antral and duodenal dog perfusates consists of the ordinary tetradecapeptide somatostatin and of a larger somatostatin molecule containing \sim 28 aminoacids in about equal proportions (to be published). The big type of somatostatin has been isolated from porcine intestinal mucosa (Pradayrol et al 1979). Thus it is highly likely that

- FRUTA, H. & KOBAYASHI, H. 1971 Experimentally induced gastric release in the endocrine cells of dogs. *Proc. 1st Int. Z. Zellforsch.* 116: 57-60.
- GUSTAVSSON, S. & LUNDQUIST, O. 1978. Participation of atrial somatostatin in the local regulation of gastric release. *Acta Endocrinologica* 88: 339-348.
- IBEDA, T., TAKAHARA, J., KAWANISHI, K., KAWAMURA, K., GOTO, A., OGAWA, N. & OFUKU, T. 1978. Direct inhibitions of basal and arginine-stimulated plasma gastrin secretion by somatostatin in anesthetized dogs. *Endocrinol. Japan* 33: 379-386.
- KJERNSSON, C., WISEN, O., KOLLBERG, B., EFENDIC, S. & UYNÄS-WALLENSTEN, K. 1978. Effect of intragastrically administered somatostatin on the basal and pentagastrin-stimulated gastric secretion in man. *Acta Physiol. Scand.* 104: 23-34.
- KUHT, R. F., FIDMAN, GREEN, R. G. & VINIK, A. J. 1973. In vivo studies of gastrin into pyloric pouch. *Br. J. Surg.* 65: 118-120.
- KONTUREK, S. J., TASLER, J., CIESZKOWSKI, B. S., COY, D. H. & SCHALLY, A. V. 1976. Effect of growth hormone release inhibiting hormone on gastric secretion, mucosal blood flow and plasma gastrin. *Gastroenterology* 70: 757-761.
- LARSSON, L. J., GÖLTHERRMANN, N., DE MÄGTER, L., REHFELD, J. F. & SCHWARTZ, T. V. 1978. Somatostatin cell processes as peptidyls in pancreatic secretion. *Science* 205: 1393-1395.
- LE ROITH, D., VINIK, A. J., EPSTEIN, S., BARON, P., OLKENITZKY, M. N., PIMSTONE, B. I. 1975. Somatostatin and serum gastrin in normal subjects and in patients with pernicious anemia, chronic liver and renal disease. *S. Afr. Med. J.* 69: 360-364.
- LEIBOW, O. 1973. Increased plasma gastrin level in connection with inhibition of gastric acid responses to stimulating following bolus perfusion with acid in dogs. *Scand. J. Gastrointest.* 10: 773-777.
- LEIBOW, O., SIVON, J., YALOW, R. S. & BERSON, S. A. 1972. Plasma gastrin and gastric responses to sham feeding and feeding in dogs. *Gastroenterology* 63: 51-59.
- OLSSON, L. 1963. Significance of vagal release of gastrin during the various phases of gastric secretion in dogs. *Gastroenterology* 44: 463-468.
- PRADYROL, L., CHAYVIALLE, L. A., MUTT, V. & KUBET, A. 1979. Isolation of porcine intestinal peptide rich C-terminal somatostatin. *Gastroenterology* 76: 1230.
- REITS, S., DOLLINGER, H. C., VON BERGER, L., SCHLEGEL, W., SCHRODER, K. E. & REIFFER, E. F. 1973. Effects of somatostatin on gastric secretion and gastrin release in man. *Digestion* 15: 25.
- REHFELD, J. F. & UYNÄS-WALLENSTEN, K. 1978. Cellular and secreted gastrins in cat and dog. Evidence for biosynthetic relationship between the large molecular forms of gastrin and heptadecapeptide gastrin. *Br. J. Physiol.* 283: 379-396.
- RUFENER, C., DUBOIS, M. P., MALAISSE LAGAE, F. & ORCI, L. 1975. Immunofluorescent reactivity to somatostatin in the gastrointestinal mucosa of the dog. *Diabetologia* 11: 371-374.
- SCHIAFFALITZKY DE MUCKADELL, O. B. & FAHRENKRUG, J. 1978. Role of secretin in man. In: *Gut hormones* (ed. S. R. Bloom), pp. 197-200. Churchill & Livingstone.
- SCHIRUPF, E., VATN, M. H., HANSEN, K. F., SEMB, L. S. & NYREN, J. 1976. Somatostatin inhibits insulin-stimulated gastrin release and gastric secretion of acid, pepsin and intrinsic factor (IF) in duodenal ulcer patients. *Scand. J. Gastrointest.* 11: 517-520.
- SCHUSDZARRA, V., HARRIS, V., CONLAN, M., ARIMURA, A. & UNGER, R. 1978. Pancreatic and gastric somatostatin release in response to intragastric and intraduodenal octreotide and HCl in the dog. *J. Clin. Invest.* 62: 509-518.
- THOMAS, J. E. 1942. A simplified procedure for preparing an improved Pavlov pouch. *Proc. Soc. Exp. Biol. (N.Y.)* 50: 58-61.
- THOMPSON, J. C. 1966. The question of an atrial chalone. In: *Gastrin* (ed. M. J. Grossman), pp. 193-228. L.A. University of California Press.
- UYNÄS, B. 1971. Role of duodenin in inhibition of gastric acid secretion. *Scand. J. Gastrointest.* 6: 113-125.
- UYNÄS-WALLENSTEN, K. 1977. The occurrence of gastrin in gastric juice, atrial secretions and atrial perforates of anesthetized cats. *Gastroenterology* 73: 487-491.
- UYNÄS-WALLENSTEN, K. 1978. Effect of atrial pH on gastrin release in anesthetized cats. *Acta Physiol. Scand.* 104: 386-393.
- UYNÄS-WALLENSTEN, K. 1978. Vagal release of atrial hormones. In: *Gut hormones* (ed. S. R. Bloom), pp. 389-393. Churchill & Livingstone.
- UYNÄS-WALLENSTEN, K. 1980. Luminal secretion of gut peptides. In: *Clinics in gastroenterology* (ed. W. Creutzfeldt), W. B. Saunders Co. Ltd., London, pp. 543-553.
- UYNÄS-WALLENSTEN, K., EFENDIC, S. & JOHANSSON, C. 1980. Intraluminal secretion of gastrointestinal hormones. The entero-angular axis (ed. W. Creutzfeldt), S. Karger AG, Basel, pp. 65-71.
- UYNÄS-WALLENSTEN, K. & EFENDIC, S. 1978. Release of gastrointestinal hormones into the duodenal lumen of cats. *Horm. Metab. Res.* 10: 173-174.
- UYNÄS-WALLENSTEN, K., EFENDIC, S. & LUFT, R. 1977. Inhibition of vagally induced gastrin release by somatostatin in cats. *Horm. Metab. Res.* 9: 120-123.
- UYNÄS-WALLENSTEN, K., EFENDIC, S. & LUFT, R. 1977. Vagal release of somatostatin into the atrial lumen of cats. *Acta Physiol. Scand.* 9: 126-128.
- UYNÄS-WALLENSTEN, K., EFENDIC, S. & LUFT, R. 1978. Release of somatostatin into the atrial lumen of cats. *Metabolism* 27 (9), Suppl. 1.
- UYNÄS-WALLENSTEN, K., LUNDBERG, J. & EFENDIC, S. 1978. Dopaminergic control of atrial gastrin and somatostatin release. *Acta Physiol. Scand.* 103: 343-345.

~2.2 at which pH level a much weaker inhibitory effect occurred (Andersson & Olbe 1963).

It cannot be established from the present data by which route the somatostatin reaches the G-cells. If we assume that the intraluminal secretion reflects a high somatostatin concentration in the mucosa, somatostatin might quite simply reach the G-cells by interepithelial diffusion, the inhibition of gastrin release thus being an example of a paracrine mode of action. However, the axonlike protrusions of antral D-cells approaching the G-cells described by Larsson et al. (1979) indicate that more specific anatomical relationships between somatostatin and gastrin cells might be involved in the inhibitory process.

The mechanism by which HCl activates the release of somatostatin is not known. Most endocrine cells of the antropyloric region are of the open type, i.e. they have an ending provided with microvilli protruding into the lumen. HCl possibly activates the release of somatostatin by acting directly on these microvilli of the D-cells. Somatostatin was also released from cells in the ileum and the specificity of the HCl induced peptide release must therefore be questioned. In fact several peptides are released in response to duodenal acidification. Secretin and VIP are released into the bloodstream following instillation of acid into the duodenum (Schaffalitzky de Muckadell & Fahrenkrug 1978; Ebeid et al. 1978) and small amounts of VIP and CCK are released by acid into the dog bulbar perfusates (Uvnäs-Wallensten & Fahrenkrug 1978; Uvnäs Wallensten & Rehfeld unpublished). It is possible that HCl activates the release of all peptides stored in endocrine cells of the open type (i.e. cells which are in direct contact with the lumen). The specificity of the pH controlled gastric acid inhibitory mechanism located to the antropyloric region might quite simply be determined by anatomical restrictions as to where a low pH may occur together with endocrine cells of the open type. These criteria are only filled in the antrum and duodenal bulb.

Such a general activation of the peptide release from endocrine cells induced by acid might explain our apparently paradoxical finding of a gastrin release in response to acidification of the antrum. Maybe 0.1 M HCl activates gastrin release as potentially as it activates somatostatin release but the smaller gastrin release observed in comparison to the output of somatostatin in spite of the larger

population of G-cells than of D-cells might be due to the fact that the G-cells are largely inhibited by the high local levels of somatostatin.

In this context it is of interest to mention, the ratio between the amounts of somatostatin and gastrin found in human gastric instillates (slightly alkaline) was 4-fold higher in patients with chronic peptic ulcer disease than in controls (Wärén et al. in press). Thus the rate of release of these peptide and maybe also the rate of peptide synthesis might be affected by longstanding variations of the intraluminal pH.

This study has been supported by grants from Norden-insulin fond, Åke Wibergs stiftelse, Hedlund stiftelse and the Swedish Medical Research Council (project No. 1907). The skilful technical assistance of Mrs Ing Brita Forsman is gratefully acknowledged.

REFERENCES

- ALBINUS M, BLAIR E L, GRUND E R, REED J D, SAUNDERS D J, GOMEZ PAN A, SCHALLY A V & BESSER, G M 1973 The mechanism where by growth hormone-releasing hormone (somatostatin) inhibits food stimulated gastric acid secretion in the cat. *Agents and Actions* 5: 306-310.
- ANDERSSON S 1960 Antrum-duodenum regionens betydelse för syntetisationen i mag-tarmkanalen. Dissertation (in Swedish). Stockholm.
- ANDERSSON S & NILSSON O 1974 Appearance of gastrin in perfusates from the isolated gastric antrum of dogs. *Scand J Gastroenterol* 9: 619-621.
- ANDERSSON S & OLBE L 1964 Inhibition of gastric acid response to sham-feeding in Pavlov pouch dogs by acidification of antrum. *Acta Physiol Scand* 61: 55-64.
- BARROS A A J, BLOOM S R & BARON J H 1978 Inhibition by somatostatin (growth-hormone releasing-inhibiting hormone) (GHI RH) of gastric acid and pepsin and G-cell release of gastrin. *Gut* 19: 315-320.
- BLOOM S R, MORTIMER C H, THORNER M O, BESSER G M, HALL R, GOMEZ PAN A, ROY U M, RUSSEL R, COY P H, KASTIN A J & SCHALLY A V 1974 Inhibition of gastrin and gastric acid secretion by growth hormone releasing inhibiting hormone. *Lancet* 9: 1106-1109.
- EBEID A M, ESCOURROU J, MURRAY F & FISCHER J E 1978. Pathophysiology of VIP I in Gut hormones (ed S R Bloom) pp 479-483. Churchill and Livingstone.
- EFENDIC S, ENGMANN F, NYLEN A, UVNAS-WALLENSTEN A & LUFT R 1980 Glucose and a sulphoprotein interaction in the release of insulin, glucagon and somatostatin from isolated perfused rat pancreas.

- MUTA, R. & KOBAYASHI, S. 1971 Experimentally induced gastric release in the endocrine cells of dogs. *Placenta* 22 (Suppl. 1) 62-69.
- OLSSON, S. & LUNDQVIST, G. 1978 Paracrine action of gastrin on the local regulation of gastric release. *Acta Endocrinologica* 88, 339-348.
- OSOTA, T., TAKAHARA, J., KAWANISHI, K., KAWAMURA, K., GOTO, A., OGAWA, N. & OFUKU, T. 1976 Direct inhibitions of basal and pyloric induced plasma gastrin secretion by somatostatin in anesthetized dogs. *Endocrinol Japan* 23 (3) 35-36.
- RIANSSON, C., NYSEN, H., KOLLBERG, B., EFENDIC, S. & UVNÄS-WALLENSTEN, K. 1978 Effects of subcutaneously administered somatostatin on the basal and pentagastrin-stimulated gastric secretion in man. *Acta Physiol Scand* 104, 232-244.
- ROBERT, M. F., FIDDIAN-GREEN, R. G. & VIVIK, A. J. 1976 In vivo studies of gastric base human gastric juice. *Br J Surg* 63 (11) 41-49.
- ROTHLIEB, S. J., TATLER, J., CIESZKOWSKI, S. S., COY, D. H. & SCHALLY, A. V. 1976 Effect of pyloric hormone release inhibiting hormone on gastric secretion, mucosal blood flow and serum gastrin. *Gastroenterology* 70, 737-741.
- ULSSON, L. L., GÖLTERMANN, H., DE MAGESTER, L., REHFELD, J. & SCHWARTZ, T. V. 1979 Somatostatin cell processes in parietal cells for pancreatic secretion. *Science* 205 (4503) 1393-1394.
- LE NOBLE, D., VIVIK, A. J., EPSTEIN, S., BAUM, P., OLKENITZKY, M. N. 1981 STONE, B. L. 1975 Somatostatin and serum gastrin: normal subjects and in patients with pernicious anemia, chronic liver and renal disease. *S Afr Med J* 69, 1441-1444.
- ULSSON, O. 1975 Increased plasma gastrin levels in connection with inhibition of gastric acid responses to stimulation following balloon perfusion with acid in dogs. *Scand J Gastrointest* 10, 773-777.
- ULSSON, G., SIMON, J., YALOW, R. S. & BERSON, S. A. 1972 Plasma gastrin and gastric acid responses to sham feeding and feeding in dogs. *Gastroenterology* 63, 51-59.
- ULSSON, L. 1983 Significance of vagal release of gastrin during the nervous phase of gastric secretion in dogs. *Gastroenterology* 44, 463-468.
- PLADYNYL, L., CHAYVALLIE, L. A., MUTT, V. & REET, A. 1979 Isolation of porcine intestinal peptide, D-C-terminal somatostatin. *Gastroenterology* 76, 1120.
- LUFT, R., DOLLINGER, H. C., VON BERGER, L., SCHLEGEL, W., SCHRODER, K. E. & REIFFER, E. F. 1975 Effects of somatostatin on gastric secretion and gastrin release in man. *Digestion* 15, 25.
- REIFFER, J. F. & UVNÄS-WALLENSTEN, K. 1978 Cellular and secreted gastrin in cat and dog. Evidence for a biosynthetic relationship between the large molecular forms of gastrin and heptadecapeptide gastrin. *Br J Physiol* 283, 379-396.
- RUFENER, C., DUBOIS, M. P., MALAISSE LAGAE, F. & ORCI, L. 1975 Immunofluorescent reactivity to somatostatin in the gastrointestinal mucosa of the dog. *Diabetologia* 11, 31-324.
- SCHAFFLITZKY, DE MUCKADELL, O. B. & FAHRENBACH, J. 1978 Role of secretin in man. In: *Gut hormones* (ed. S. R. Bloom), pp. 197-200. Churchill & Livingstone.
- SCHRUMPF, E., VATN, M. H., HANSEN, A. F., SEMB, L. S. & MYREN, J. 1976 Somatostatin inhibits insulin stimulated gastrin release and gastric secretion of acid, pepsin and intrinsic factor (IF) in duodenal ulcer patients. *Scand J Gastrointest* 11, 517-520.
- SCHUSDZARRA, V., HARRIS, V., CONLAN, M., ARIMURA, A. & UNDER, R. 1978 Pancreatic and gastric somatostatin release in response to intragastric and intraduodenal nutrient and HCl in the dog. *J Clin Invest* 61, 509-518.
- THOMAS, J. E. 1941 A simplified procedure for preparing an improved Pavlov pouch. *Proc Soc Exp Biol (NY)* 40, 58-61.
- THOMPSON, J. C. 1966 The question of an antral chalone. In: *Gastrin* (ed. M. J. Grossman), pp. 191-228. L.A. University of California Press.
- UVNÄS, B. 1971 Role of duodenal inhibition of gastric acid secretion. *Scand J Gastrointest* 11, 113-115.
- UVNÄS-WALLENSTEN, K. 1977 The occurrence of gastrin in gastric juice: antral secretions and antral perfuates of anesthetized cats. *Gastroenterology* 73, 487-491.
- UVNÄS-WALLENSTEN, K. 1978 Effect of intragastric pH on gastrin release in anesthetized cats. *Acta Physiol Scand* 104, 366-393.
- UVNÄS-WALLENSTEN, K. 1978 Vagal release of antral hormones. In: *Gut hormones* (ed. S. R. Bloom), pp. 389-393. Churchill & Livingstone.
- UVNÄS-WALLENSTEN, K. 1980 Luminal secretion of gut peptides. In: *Clinics in gastroenterology* (ed. W. Creutzfeldt), W. B. Saunders Co Ltd, London, pp. 445-553.
- UVNÄS-WALLENSTEN, K., EFENDIC, O. & JOHANSSON, C. 1980 Intraluminal secretion of gastrointestinal hormones. The entero-insular axis (ed. W. Creutzfeldt). In: *Karger AO Basel*, pp. 65-71.
- UVNÄS-WALLENSTEN, K. & EFENDIC, S. 1978 Release of gastrointestinal hormones into the duodenal lumen of cats. *Horm Metab Res* 10, 173-174.
- UVNÄS-WALLENSTEN, K., EFENDIC, S. & LUFT, R. 1977 Inhibition of vagally induced gastrin release by somatostatin in cats. *Horm Metab Res* 9, 120-123.
- UVNÄS-WALLENSTEN, K., EFENDIC, S. & LUFT, R. 1977 Vagal release of somatostatin into the antral lumen of cats. *Acta Physiol Scand* 9, 126-128.
- UVNÄS-WALLENSTEN, K., EFENDIC, S. & LUFT, R. 1978 Release of somatostatin into the antral lumen. *Febs. Metabolism* 27 (5) Suppl. 1.
- UVNÄS-WALLENSTEN, K., LUNDBERG, J. & EFENDIC, S. 1978 Dopaminergic control of antral gastrin and somatostatin release. *Acta Physiol Scand* 103, 343-345.

- UVNÄS-WALLENSTEN K, EFENDIC S, LARSSON I & JOHANSSON C. 1979 Phosphate buffer stimulates somatostatin release into the antral lumen of anaesthetized cats. *Scand J Gastroent* 14: 797-800.
- WALSH J, RICHARDSON C T & FORDTRAN J. 1975 pH dependence of acid secretion and gastrin release in normal and ulcer subjects. *J Clin Invest* 55: 462-466.
- WHEELER M H. 1974 Progress report: inhibition of gastric secretion by the pyloric antrum. *Gut* 15: 43.
- WISÉN O, UVNÄS-WALLENSTEN K, EFENDIC S & JOHANSSON C. 1980. Release of gastrin and somatostatin into the gastric lumen of healthy subjects and patients with duodenal ulcer or achlorhydria. *Acta Physiol Scand* 108: 297-300.

Pre- and postjunctional effects of prostaglandin E₂ and prostaglandin synthetase inhibitors and atropine on cholinergic neurotransmission in guinea pig ileum and bovine iris

LARS GUSTAFSSON, PER HEDQVIST and GÖSTA LUNDGREN

Department of Physiology and Department of Toxicology, Karolinska Institute, Stockholm, Sweden

GUSTAFSSON L, HEDQVIST P & LUNDGREN G. Pre- and postjunctional effects of prostaglandin E₂, prostaglandin synthetase inhibitors and atropine on cholinergic neurotransmission in guinea pig ileum and bovine iris. *Acta Physiol Scand* 1980; 110: 401-411. Received 12 March 1980. ISSN 0001-6777. Department of Physiology and Department of Toxicology, Karolinska Institute, Stockholm, Sweden.

The effects of prostaglandin E₂, arachidonic acid, prostaglandin synthetase inhibitors and atropine on cholinergic neuromuscular transmission were examined in isolated guinea pig ileum longitudinal muscle and bovine iris sphincter muscle. Prostaglandin E₂ and arachidonic acid markedly enhanced contraction responses induced by nerve stimulation. In addition, prostaglandin E₂ enhanced contraction responses to acetylcholine and direct muscle stimulation, apparently to the same extent as those in nerve stimulation. Prostaglandin synthetase inhibitors (indomethacin, meclofenamic acid and enoxatetraacetic acid) very effectively reduced contraction responses to nerve stimulation or acetylcholine, and they abolished the stimulant effect of arachidonic acid. Basal and nerve-induced efflux of acetylcholine from the everted bladder as measured by mass fragmentography was unaffected by prostaglandin E₂, but diminished slowly during indomethacin. Subsequent prostaglandin administration caused slight increase of nerve-induced release of acetylcholine. Atropine markedly increased overflow of acetylcholine from stimulated preparations of both types. This indicates that muscarinic receptors, causing diminished acetylcholine release in everted bladder, may be present also at postganglionic terminals. During atropine, an effect of prostaglandin E₂ on acetylcholine release could still not be seen. Thus, using physicochemical determination of acetylcholine we could not verify earlier reports, employing bioassay, claiming enhanced release of transmitter during prostaglandin E₂ treatment. However, it seems likely that prostaglandin E₂ takes part in the regulation of contractility and tone of the smooth muscle cells.

Key words: Acetylcholine, atropine, bovine iris, choline gas chromatography-mass spectrometry, guinea pig ileum, neuromuscular transmission, prostaglandins, smooth muscle

Prostaglandin (PGs) of the F and F series contract and PG synthetase inhibitors relax smooth muscle in even cholinergically innervated tissues such as the intestine and iris (Bergstrom et al 1959; Dixon, Rainsell & Willis 1972; Ferreira, Herman & Vane 1972). Since PGs are released from these tissues, both basally and in response to a variety of stimuli (Pomeroy 1973; Botting & Salzman 1974), a possible role of locally formed PGs as modulators of smooth muscle tone has been envisaged (Bennett & Pomeroy 1971; Ferreira, Herman & Vane 1976). Apart from directly stimulating the smooth mus-

cle cells PGs may also influence the cholinergic neuroeffector transmission in guinea pig ileum (Harry 1968) and bovine iris (Gustafsson, Hedqvist & Lagercrantz 1975). Since PGs enhance contraction responses both to acetylcholine (ACh) and nerve stimulation (Harry 1968; Baum & Shropshire 1971; Hadházy Illés & Knoll 1973; Illés, Vizi & Knoll 1974; Gustafsson et al 1975) a postjunctional potentiating effect on cholinergic transmission may be present, although results denying this have been presented (Hall, O'Neill & Sheehan 1975).

The hazards in interpretation of differential quan-

- UVNÄS-WALLENSTEN K, EFENDIC E, LARSSON I & JOHANSSON C 1979 Phosphate buffer stimulates somatostatin release into the antral lumen of anaesthetized cats. *Scand J Gastroent* 14: 797-800.
- WALSH J, RICHARDSON C, T & FORDTRAN J 1975 pH dependence of acid secretion and gastrin release in normal and ulcer subjects. *J Clin Invest* 55: 462-466.
- WHEELER, M. H. 1974 Progress report inhibition gastric secretion by the pyloric antrum. *Gut* 4: 43.
- WISÉN O, UVNÄS-WALLENSTEN K, EFENDIC S & JOHANSSON C. 1980 Release of gastrin and somatostatin into the gastric lumen of healthy subjects and patients with duodenal ulcer or achlorhydria. *Acta Physiol Scand* 108: 797-800.

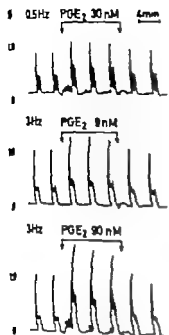


Fig. 1 Isolated longitudinal muscle of the guinea pig ileum. Effect of PGE_2 on phasic and tonic contraction responses to transmural stimulation (0.5 or 3 Hz, 1 sec) for 1 sec at 1 min intervals. Wash after each application of the prostaglandin. Isometric recording.

effect was rapid in onset, vanished quickly when the preparation was washed, and occurred with PGE_2 concentrations (3–100 nM) and stimulation frequencies (0.5–3 Hz) used when release of acetylcholine (ACh) was determined. In both tissues PGE_2 potentiated contraction responses to ACh, as well as those induced by direct muscle stimulation after prior treatment with tetrodotoxin (0.1–0.3 μ M) (Figs 1, 3). These results strongly suggest that PGE_2 enhanced also neurogenic responses by a prejunctional action. In fact, PGE_2 (0.6 nM) increased the contraction response to direct muscle

Table 1 Percent increase of contraction responses to transmural nerve stimulation (3 Hz, 1 ms, 15 pulses at 1 min intervals) 10 min after administration of arachidonic acid (AA) in bovine iris sphincter

Mean values \pm S.E. from 5 experiments at each dose level. All values significant at least at the 5% level

AA μ M	% increase
10	28.9
30	46.16
100	11.33

stimulation in the bovine iris by $47 \pm 15\%$ ($P < 0.05$, $n=6$) which does not differ from previously observed enhancement of neurogenic responses in this tissue (cf. Gustafsson et al. 1975).

The prostaglandin precursor arachidonic acid dose-dependently enhanced the contraction response to transmural nerve stimulation in guinea pig ileum and bovine iris sphincter (Table 1). Administration of either of two prostaglandin synthetase inhibitors, meclofenamic acid and indomethacin ($\sim 3 \mu$ M), annulled the effect of arachidonic acid without blocking the stimulant action of PGE_2 (Figs 4, 5). This indicates that newly formed prostaglandin rather than arachidonic acid itself was responsible for the stimulant effect.

Indomethacin and meclofenamic acid ($\sim 6 \mu$ M) as well as the substrate analogue eicosatetrynoic acid (ETA) (10–70 μ M), depressed the contraction response to transmural nerve stimulation (Figs 4, 6, 7). They also depressed the contraction response to exogenous ACh (Fig. 3). Inhibition of neurogenic responses developed rapidly in the guinea pig ileum, being manifest within 30 s and reaching its maximum after 5–7 min, whereas the effect developed at a somewhat slower rate in the iris. In both tissues the end result was, however, the same.

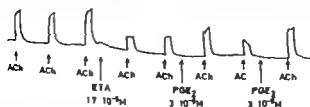


Fig. 2 Isolated bovine iris sphincter muscle. Reversal by PGE_2 of the inhibitory effect of eicosatetrynoic acid (ETA) on contraction responses to standard doses (3.3 μ M) of acetylcholine (ACh). Wash at dots.

tative effects of PGs on the responses to exogenous agonist and to nerve stimulation respectively have been emphasized (Baum & Shropshire 1971). However, inferring from smaller effect on ACh responses than on responses to transmural stimulation PGs have been claimed to enhance and PG synthesis inhibitors to decrease transmitter release from cholinergic nerves in guinea pig ileum (Ehrenpreis, Greenberg & Belman 1973; Kadlec, Masek & Seferna 1974; Bennett, Eley & Stockley 1975).

Attempts to disclose a prejunctional effect of PGs on cholinergic neurotransmission by measurement of ACh release have led to conflicting results. Thus on the basis of bioassay of released ACh PGs have been reported to lack effect (Hadházy et al 1973; Illés et al 1974) or to enhance (Radmanovic 1975; Kadlec, Masek & Seferna 1978) the secretion of transmitter in response to nerve stimulation. Likewise PG synthesis inhibitors have been reported either to depress (Kadlec et al 1974; Hall et al 1975) or to have no effect (Botling & Salzmann 1974; Hazra 1975) on induced secretion of ACh.

In view of these conflicting results it was decided to re-investigate pre- and postjunctional effects of PGs and PG synthesis inhibitors on cholinergic neurotransmission using gas chromatography-mass spectrometry (GC/MS) for determination of secreted ACh and choline. Experiments were performed with both the guinea pig ileum and the bovine iris sphincter to differentiate between pre- and postganglionic mechanisms. It was further of interest to make experiments taking in account a possible auto-inhibition of ACh release in eserized tissue (cf. Szerb 1979) and experiments were thus also performed in the presence of atropine. A preliminary account of part of the results has been presented elsewhere (Hedqvist et al 1980).

METHODS

General procedure

Male guinea pigs (250–500 g) were stunned and bled. Strips of plexus-containing longitudinal muscle from the distal part of ileum were isolated according to Paton & Vizi as described previously (Gustafsson et al 1978). Whole segments of ileum with tied ends were prepared as well and stimulated according to Flinkelman (Gustafsson et al 1978) for activation of mesenteric adrenergic nerves. Also used was the isolated sphincter muscle from bovine preputia (Gustafsson et al 1975). The smooth muscle preparations were mounted in 5 ml organ baths containing Tyrode's solution of the following composition (conc in

mM): Na 149.2, K 4.7, Ca 1.8, Mg 0.5, Cl 144.2, H₂PO₄ 11.9 and glucose 5.5. In some ileum experiments the Tyrode's was replaced by a Krebs' solution according to Kadlec et al (1974) (conc in mM): Na 135.4, K 5.9, 2.5, Mg 1.2, Cl 133.3, HCO₃ 15.4 and glucose 11.5). Bath fluid was continuously gassed with 5% CO₂ in O₂, was kept at 37°C. Biphasic single pulses or trains of pulses (duration 1 msec, supramaximal voltage, specifically activating the nerves) were delivered by means of a platinum wire electrodes along the walls of the bath, a Grass S88 stimulator. Muscle tone and contractions induced by drugs and transmural nerve stimulation were studied at a load of 0.1–0.5 g and recorded either isotonically (Harvard Apparatus smooth muscle transducer type 356) or isometrically (Grass FT 03C transducer) and displayed on an ordinate writer (Harvard Electronik 194) or a polygraph (Grass 5P). Figures are isotonic recordings unless otherwise stated.

Release of ACh from the preparations was determined essentially as described by Gustafsson et al (1978). Briefly the preparation was mounted in a 1 ml bath perfused with Tyrode's containing 3 µM eserine. Preparation was transmurally stimulated (0.5–1 H msec, supramaximal voltage) for 5 min at 20 min later. Muscle tone and induced contractions were recorded isometrically. The bath fluid was collected at 5 min intervals immediately chilled, acidified and stored at -20°C until analyzed for ACh and choline by GC/MS according to the method of Karlén et al (1974).

Drugs

Acetylcholine hydrochloride (Sigma Co. St. Louis, USA), arachidonic acid (Sigma), atropine sulfate (Sigsco, Switzerland), 6-aminocaproic acid (P. Hoffmann-La Roche & Basle, Switzerland), eserine sulfate (Sigma), guanine sulfate (Ciba-Geigy AG, Basel, Switzerland), 6-dopamine (Sigma), indomethacin hydrochloride (M. Sharp and Dohme, Rahway, New Jersey, USA), prostaglandin E free acid (a gift from Dr J. Pike, The Upjohn Co., Kalamazoo, Michigan, USA), sodium metoheptate (Parke Davis & Co., Detroit, Michigan, USA), tetrodotoxin (Sigma). Deuteriated (D₃) ACh and choline derivatives were synthesized at the Department of Toxicology, Karolinska Institutet. All chemicals used were of reagent grade.

Statistics

Experimental data were expressed as means ± S.E. Statistical significance was tested according to Student's *t* for paired or unpaired variates.

RESULTS

In harmony with previous results (Baum & Shropshire 1971; Gustafsson et al 1975) PGE₂ enhanced the twitch response to transmural nerve stimulation in guinea pig ileum and bovine iris sphincter. Transmural contractions induced by prolonged stimulus periods were also enhanced by PGE₂ (Fig. 1).

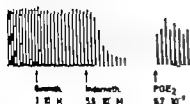


Fig. 6 Isolated longitudinal muscle of the guinea pig ileum. Contraction responses to transmural stimulation (3 Hz, 15 pulses, 1 ms) at 1 min intervals. Reversal by PGE_2 of the inhibitory effect of indomethacin during adrenergic block with guanethidine.



Fig. 7 Isolated bovine iris sphincter muscle. Contraction responses to transmural stimulation (3 Hz, 15 pulses, 1 ms) at 1 min intervals. Reversal by PGE_2 of the inhibitory effect of indomethacin.

also is inhibitors of neurogenic responses in the presence of the adrenergic neurone blocker guanethidine ($0.1 \mu\text{M}$). This occurred independently of whether normal Tyrode's or the Krebs solution of Kadic et al. was used (Fig. 6). In the above mentioned concentration guanethidine blocked completely the adrenergic transmission in the guinea pig ileum prepared according to Finkelman. Furthermore, in two experiments guinea pigs were densely sympathectomized (Sachs & Jonsson 1973) with 6-hydroxy-dopamine (25 mg/kg intracardially on two consecutive days). This treatment reduced cardiac catecholamine levels by at least 90%. Yet, indomethacin inhibited the nerve-induced contraction response in the ileum exactly as in preparations from untreated animals.

In the non-exerized guinea pig ileum transmural nerve stimulation, 3–10 Hz for 5 min in the absence or presence of atropine ($1.4 \mu\text{M}$) evoked a minute

outflow of ACh (less than 1 pmol/ml) which could not be accurately quantified by GC/MS. Basal outflow of ACh was always below the level of detection.

In the eserized tissue ($3 \mu\text{M}$) transmural nerve stimulation evoked a reproducible and frequency dependent outflow of ACh well above the resting level (Fig. 9 Table 1). Choline also accumulated in the bath medium, but nerve stimulation did not change the rate of accumulation (Table 2). Notably virtually the same amounts of choline were found in the medium surrounding the non-eserized ileum and again there was no difference between resting and stimulation periods.

Addition to the bath of atropine in concentrations ranging between 1.4 nM and $1.4 \mu\text{M}$ consistently and dose-dependently increased the stimulation-evoked release of ACh. A further increase in atropine concentration to $1.4 \mu\text{M}$ did not cause any additional

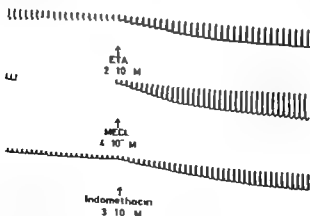


Fig. 8 Isolated bovine iris sphincter muscles. Contraction responses to transmural stimulation (3 Hz, 15 pulses, 1 ms) at 1 min intervals. After more than one hour at 37°C tone had developed spontaneously and was effectively reversed by eicosatetraynoic acid (ETA), meclofenamic acid (MECL) or indomethacin.

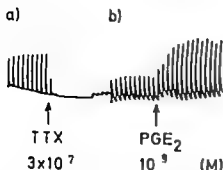


Fig 3 Isolated bovine iris sphincter. Potentiation by PGE_2 of contraction responses to transmurial stimulation during tetrodotoxin (TTX) treatment (a) 3 Hz, 1 ms, 15 pulses at 1 min intervals (b) 40 ms pulse duration, contractions amplified $\times 4$.

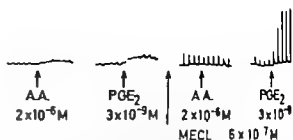


Fig 4 Isolated longitudinal muscle of the guinea pig ileum. Contraction responses to transmurial stimulation (1 Hz, 2 pulses, 1 ms) at 1 min intervals. Effect of arachidonic acid (AA) or PGE_2 before and during administration of meclofenamic acid (MECL). Wash between registrations.

ie a very marked or virtually complete inhibition of the contraction response which was highly resistant to washing of the preparation. Subsequent administration of PGE_2 in very low concentrations (0.6–6 nM) promptly increased the contraction response to transmurial nerve stimulation (Figs 4, 6, 7). Using slightly higher concentrations of PGE_2 (10–60 nM) normal or supranormal responses were consistently obtained.

A conspicuous feature of the bovine iris sphincter is a progressive rise in intrinsic tone occurring after approximately 1 h of incubation in Tyrode's solution (Gustafsson et al 1975). The increase in tone seems to be paralleled by prostaglandin accumulation in the bath medium (Posner 1973). In the present study any one of three prostaglandin synthetase inhibitors, indomethacin, meclofenamic acid and eicosatetraynoic acid, annulled the ex-

pected rise in tone. They also brought an already developed tone back to its basal level (Fig 8), and subsequently followed by inhibition of the nerve-induced contraction responses. These observations suggest that endogenous PGE_2 may modulate both intrinsic tone and the neuroeffector transmission, and that the latter action requires lower concentrations of the compound. This view is supported by the effects of exogenous PGE_2 : low doses enhance contraction responses and higher doses increase the intrinsic tone.

It has been suggested that indomethacin inhibits contraction responses in the guinea pig ileum by causing increased release of noradrenaline, in turn inhibiting the ACh release and leading to reduced effector responses (Kadlec et al 1974). We have consistently failed to confirm this view. Thus, indomethacin and meclofenamic acid remained full-

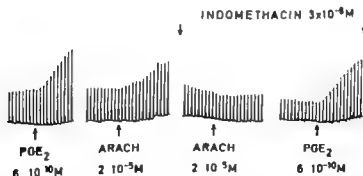


Fig 5 Isolated bovine iris sphincter muscle. Contraction responses to transmurial stimulation (3 Hz, 15 pulses, 1 ms) at 1 min intervals. Effect of PGE_2 or arachidonic acid (ARACH) before and during administration of indomethacin. Wash at dots.

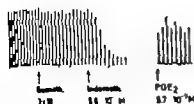


Fig. 6 Isolated longitudinal muscle of the guinea pig ileum. Contraction responses to transmural stimulation (5 Hz, 10 pulses, 1 ms) at 1 min intervals. Reversal by PGE_2 of the inhibitory effect of indomethacin during adrenergic block with phentolamine.

which is inhibitors of neurogenic responses in the presence of the adrenergic neurone blocker phentolamine ($3 \mu\text{M}$). This occurred independently of whether normal Tyrode's or the Krebs's solution of Kuffler et al. was used (Fig. 6). In the above-mentioned concentration guanethidine blocked completely the adrenergic transmission in the guinea pig ileum prepared according to Finkelman. Furthermore in the experiments guinea pigs were densely sympathectomized (Sachs & Jonsson 1974) with 6-hydroxy-dopamine (25 mg/kg intracardially on two consecutive days). This treatment reduced cardiac catecholamine levels by at least 90%. Yet, indomethacin inhibited the nerve-induced contraction response in the ileum exactly as in preparations from untreated animals.

In the non-eserinized guinea pig ileum transmural nerve stimulation, 3–10 Hz for 5 ms, in the absence or presence of atropine ($1.4 \mu\text{M}$) evoked a minute



Fig. 7 Isolated bovine iris sphincter muscle. Contraction responses to transmural stimulation (3 Hz, 15 pulses, 1 ms) at 1 min interval. Reversal by PGE_2 of the inhibitory effect of indomethacin.

outflow of ACh (less than 1 pmol/ml) which could not be accurately quantified by GC-MS. Basal outflow of ACh was always below the level of detection.

In the eserinizated tissue ($3 \mu\text{M}$) transmural nerve stimulation evoked a reproducible and frequency dependent outflow of ACh well above the resting level (Fig. 9 Table 1). Choline also accumulated in the bath medium but nerve stimulation did not change the rate of accumulation (Table 2). Notably virtually the same amounts of choline were found in the medium surrounding the non-eserinized ileum, and again there was no difference between resting and stimulation periods.

Addition to the bath of atropine in concentrations ranging between 14 nM and $1.4 \mu\text{M}$ consistently and dose-dependently increased the stimulation-evoked release of ACh. A further increase in atropine concentration to $14 \mu\text{M}$ did not cause any additional

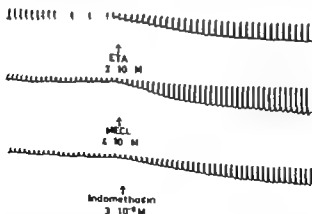


Fig. 8 Isolated bovine iris sphincter muscles. Contraction responses to transmural stimulation (3 Hz, 15 pulses, 1 ms) at 1 min intervals. After more than one hour at 37°C the tissue had developed spontaneously and was effectively reversed by elicosateiraynol acid (ETA), methoxycinnamic acid (MECL) or indomethacin.

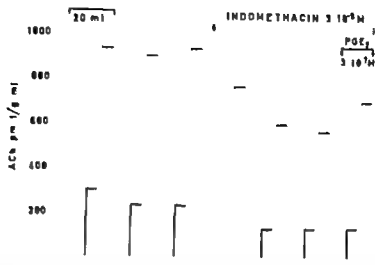


Fig. 9 Effect of indomethacin and PGE_2 on acetylcholine (ACh) release as measured by GC/MS from isolated longitudinal muscle of the guinea pig ileum. Transmural stimulation (3 Hz, 1 ms, 840 pulses) during 5 min period at 20 min intervals. Bath fluid changed every 5 min. Short columns: pre-stimulation periods; tall columns: stimulation period.

increase in ACh release (Fig. 10). Atropine ($1.4 \mu\text{M}$) substantially increased the release of ACh at both 0.5 and 3 Hz stimulation, while having no effect on basal release of the transmitter (Table 2). The nominal increase in ACh release was more marked at 3 Hz stimulation, but on a percentage basis the enhancement was virtually the same, being 710 and 184% at 0.5 and 3 Hz, respectively. Atropine did not significantly affect choline release from the resting or stimulated guinea pig ileum (Table 2).

Closely similar results were obtained in 3 expts. with the bovine iris sphincter. Transmural nerve stimulation at 3 Hz released 97 ± 47 pmol ACh \times $\text{g}^{-1} \times \text{min}^{-1}$. Subsequent administration of atropine ($1.4 \mu\text{M}$) increased the release of ACh to 285 ± 91 pmol \times $\text{g}^{-1} \times \text{min}^{-1}$ during the following stimulation period.

Administration of PGE_2 , 10 min before and during a subsequent 5 min stimulation at 0.5 or 3 Hz was found to have no effect on basal and stimulator-evoked release of ACh in the guinea pig ileum. The lack of effect was seen both with a PGE_2 concentration 3 nM , which normally gives rise to marked enhancement of stimulation-evoked contraction responses (cf. Fig. 1) and a 100 times higher concentration (Table 3, Fig. 11). Choline release was equally unaffected by PGE_2 in these concentrations. In the bovine iris sphincter (3 expts.) PGE_2 ($0.3 \mu\text{M}$) had no discernible effect on the release of ACh induced by 3 Hz stimulation, irrespective of being tested before or after atropine treatment ($1.4 \mu\text{M}$).

In view of indomethacin effectively inhibiting the contraction response to nerve stimulation, its effect

Table 2. Isolated longitudinal muscle of the guinea pig ileum

Effect of $1.4 \mu\text{M}$ atropine on acetylcholine (ACh) and choline (Ch) release immediately before (basal) and during (stim) transmural nerve stimulation (0.5 or 3 Hz, 1 ms, 140 or 840 pulses, respectively). Bath fluid collected at 5 min intervals. ACh and Ch determined by GC/MS and expressed in pmol/g \times min (tissue wet weight). (Mean values \pm S.E., $n = 3$, $P < 0.05$, $n =$ number of expts.)

	Control		Atropine $1.4 \mu\text{M}$		<i>n</i>
	ACh	Ch	ACh	Ch	
Basal	166 ± 11	1017 ± 168	148 ± 51	903 ± 9	4
0.5 Hz stim	258 ± 33	1139 ± 104	536 ± 111	935 ± 100	4
Basal	228 ± 12	77 ± 9	13 ± 34	544 ± 1.5	7
3 Hz stim	143 ± 144	857 ± 71	2785 ± 35	855 ± 101	8

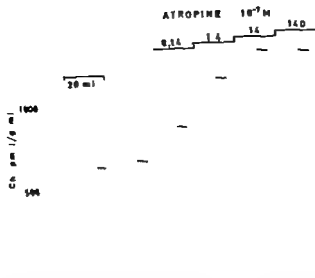


Fig. 10 Effect of atropine in decreasing concentrations on stimulation-induced acetylcholine (ACh) release as measured by GCM from isolated longitudinal muscle of the guinea pig ileum. Transmucosal stimulation (3 Hz, 1 ms, 840 pulses) during 5 min periods at 20 min intervals. Bath fluid changed every 5 min.

on ACh release was also tested in the guinea pig ileum. Indomethacin, 30 μ M, reduced basal outflow of ACh as well as release of the transmitter induced by nerve stimulation at 0.5 and 3 Hz (Table 3 Fig. 9). The inhibition developed remarkably slowly

reaching its maximum after 40 to 60 min, and it did not exceed 40%. In 4 out of 5 expts subsequent administration of PGE_2 0.3 μ M caused a minor increase of ACh release during nerve stimulation.

Table 3 Effect of PGE_2 , atropine and indomethacin on GCMs-determined efflu of ACh induced by transmucosal nerve stimulation (0.5–3 Hz, 1 ms) for 5 min at 20 min intervals 1 g guinea pig ileum longitudinal muscle

Values are given as means \pm S.E. as percent of control stimulation preceding drug administration. n = number of expts. Statistical evaluation according to Student's *t*-test for paired variables. In the case of PGE_2 effect in presence of atropine or indomethacin by setting atropine and indomethacin data to 100%. ATR = atropine, IND = indomethacin. n.s. = not significant.

Drug	0.5 Hz	3 Hz	0.5+3 Hz	
—	106 \pm 6 n=12	106 \pm 3 1	106 \pm 3	n.s.
PGE_2 3 nM		111 \pm 12 3		
PGE_2 300 nM	105 \pm 9 5	107 \pm 6 3	106 \pm 6	n.s.
ATR 1.4 μ M	210 \pm 28 4	189 \pm 14 8	196 \pm 13	} n.s.
ATR PGE_2 1.4 μ M 300 nM	200 \pm 45 4	193 \pm 11 3	197 \pm 24	
IND 30 μ M	68 \pm 6 3	64 \pm 6 3	65 \pm 4	} P<0.05
IND PGE_2 30 μ M 300 nM	81 \pm 11 3	80 2	81 \pm 6	

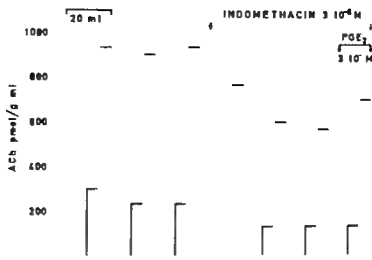


Fig. 9 Effect of indomethacin and PGE_2 on acetylcholine (ACh) release as measured by GcMs from isolated longitudinal muscle of the guinea pig ileum. Transmural stimulation (3 Hz, 1 ms, 840 pulses) during 5 min periods at 20 min intervals. Bath fluid changed every 5 min. Short columns: pre-stimulation periods; tall columns: stimulation periods.

increase in ACh release (Fig. 10). Atropine $1.4 \mu\text{M}$ substantially increased the release of ACh at both 0.5 and 3 Hz stimulation whilst having no effect on basal release of the transmitter (Table 2). The nominal increase in ACh release was more marked at 3 Hz stimulation, but on a percentage basis the enhancement was virtually the same, being 210 and 184% at 0.5 and 3 Hz, respectively. Atropine did not significantly affect choline release from the resting or stimulated guinea pig ileum (Table 2).

Closely similar results were obtained in 3 expts with the bovine iris sphincter. Transmural nerve stimulation at 3 Hz released 97 ± 42 pmol ACh \times g \times min. Subsequent administration of atropine $1.4 \mu\text{M}$ increased the release of ACh to 285 ± 91 pmol \times g \times min during the following stimulation period.

Administration of PGE_2 10 min before and during a subsequent 5 min stimulation at 0.5 or 3 Hz was found to have no effect on basal and stimulation-evoked release of ACh in the guinea pig ileum. This lack of effect was seen both with a PGE_2 concentration 3 nM which normally gives rise to a marked enhancement of stimulation-evoked contraction responses (cf Fig. 1) and a 100 times higher concentration (Table 3, Fig. 11). Choline release was equally unaffected by PGE_2 in these concentrations. In the bovine iris sphincter (7 expts) PGE_2 $0.3 \mu\text{M}$ had no discernible effect on the release of ACh induced by 3 Hz stimulation, irrespective of being tested before or after atropine treatment $1.4 \mu\text{M}$.

In view of indomethacin effectively inhibiting the contraction response to nerve stimulation, its effect

Table 2 Isolated longitudinal muscle of the guinea pig ileum

Effect of $1.4 \mu\text{M}$ atropine on acetylcholine (ACh) and choline (Ch) release immediately before (basal) and during (stim) transmural nerve stimulation (0.5 or 3 Hz, 1 ms, 140 or 840 pulses, respectively). Bath fluid collected at 5 min intervals. ACh and Ch determined by GcMs and expressed in pmol/g \times min (tissue wet weight). (Mean values \pm S.E., $n = \text{number of expts}$). $^*P < 0.05$, $^{**}P < 0.001$.

	Control		Atropine $1.4 \mu\text{M}$		
	ACh	Ch	ACh	Ch	
Basal	166 ± 11	1017 ± 168	148 ± 51	903 ± 25	4
0.5 Hz stim	258 ± 33	1139 ± 224	536 ± 85	935 ± 200	4
Basal	228 ± 12	772 ± 95	13 ± 34	544 ± 125	7
3 Hz stim	1243 ± 144	857 ± 71	2285 ± 35	855 ± 103	8

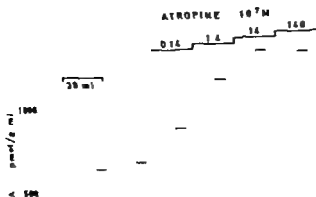


Fig. 10 Effect of atropine in increasing concentrations on stimulation-induced acetylcholine (ACh) release as measured by GcMs from isolated longitudinal muscle of the guinea pig ileum. Transverse stimulation (3 Hz, 1 ms, 840 pulses) during 5 min periods at 20 min intervals. Bath fluid changed every 5 min.

on ACh release was also tested in the guinea pig ileum. Indomethacin, 30 μ M, reduced basal outflow of ACh as well as release of the transmitter induced by nerve stimulation at 0.5 and 3 Hz (Table 3, Fig. 9). The inhibition developed remarkably slowly

reaching its maximum after 40 to 60 min, and it did not exceed 40%. In 4 out of 5 expts subsequent administration of PGE₂ 0.3 μ M caused a minor increase of ACh release during nerve stimulation.

Table 3. Effect of PGE₂, atropine and indomethacin on GcM-determined efflux of ACh induced by transverse nerve stimulation (0.5–3 Hz, 1 ms) for 5 min at 20 min intervals in guinea pig ileum longitudinal muscle

Values are given as means \pm S.E. in percent of control stimulation preceding drug administration. n = number of expts. Statistical evaluation according to Student's *t*-test for paired variables. In the case of PGE₂ effect in presence of atropine or indomethacin by setting atropine and indomethacin data to 100%. ATR=atropine, IND=indomethacin, n = not significant.

Drug	0.5 Hz	3 Hz	0.5+3 Hz	
Nil	106 \pm 6 n=12	106 \pm 3 n=12	106 \pm 3	n.s.
PGE ₂ 3 μ M		111 \pm 12 n=3		
PGE ₂ 300 nM	105 \pm 9 n=5	107 \pm 6 n=3	106 \pm 6	n.s.
ATR 1.4 μ M	210 \pm 28 n=4	189 \pm 14 n=8	196 \pm 13	} n.s.
ATR PGE ₂ 1.4 μ M 300 nM	200 \pm 45 n=4	193 \pm 11 n=5	197 \pm 24	
IND 30 μ M	68 \pm 6 n=3	62 \pm 6 n=3	65 \pm 4	} P<0.05
IND+PGE ₂ 30 μ M, 300 nM	81 \pm 11 n=3	80	81 \pm 6	



Fig 11 Effect of indomethacin and PGE_2 on acetylcholine (ACh) release as measured by GCMS from isolated longitudinal muscle of the guinea pig ileum. Transmural stimulation (3 Hz, 1 ms, 840 pulses) during 5 min periods at 70 min intervals. Bath fluid changed every 5 min. Short columns: pre-stimulation periods; tall columns: stimulation periods.

DISCUSSION

It has been suggested (Gustafsson et al 1975) that prostaglandins might operate to modulate smooth muscle responsiveness to cholinergic nerve activity. In the iris the experimental basis for this assumption being that prostaglandins are formed in the tissue (Posner 1973) and that E and F prostaglandins cause a dose-dependent enhancement of contraction responses to cholinergic nerve stimulation (Gustafsson et al 1975). The present results lend further support to this view. Thus three prostaglandin synthetase inhibitors: indomethacin, meclofenamic acid and eicosatetraynoic acid, although chemically very dissimilar (cf Flower 1974), inhibited the contraction responses to cholinergic nerve stimulation in the bovine iris sphincter muscle. Moreover, subsequent administration of PGE_2 restored normal contraction responses. In addition, it was found that the prostaglandin precursor arachidonic acid enhanced nerve-induced contraction responses, and that this effect was markedly counteracted or even annulled by prior application of indomethacin or meclofenamic acid.

In harmony with several other reports (Kadlec et al 1974; Bennett et al 1975; Ehrenpreis, Greenberg & Comaty 1976), PGE_2 enhanced and prostaglandin synthetase inhibitors reduced the contraction responses to cholinergic nerve stimulation in guinea pig ileum. Furthermore, the stimulant effect of PGE_2 was mimicked by arachidonic acid. Seemingly

PGE_2 could act on ganglionic and/or neuroeffector transmission in the ileum. However, the strikingly similar effects obtained in the iris sphincter, which is a ganglion-free preparation (cf Beutler & Stillwell 1961), would seem to indicate that in both tissues the target was the neuroeffector transmission.

It has been proposed (Kadlec et al 1974) that PGs enhance cholinergic responses in the guinea pig ileum by interfering with a suggested adrenergic control of ACh release (Beani, Bianchi & Crema 1969; Paton & Vizi 1969). The present results do not support such a mechanism, since PGE_2 and indomethacin remained fully active on cholinergic responses after adrenergic neuronal blockade (guanethidine) or chemical sympathectomy (6-OH-dopamine).

An important issue is whether the potentiating effect of PGs on the neuromuscular transmission is pre- or postjunctionally located. In both ileum and iris, PGE_2 enhanced and PG synthetase inhibition reduced contraction responses to ACh to approximately the same extent as they affected responses to nerve stimulation. In addition, PGE_2 potentiated responses to direct electrical muscle stimulation in the two preparations. Therefore, a substantial part of the PG effect must be due to a postjunctional action.

As pointed out in the Introduction, several reports have dealt with a prejunctional action of PGs and PG synthetase inhibitors on ACh release in the guinea

guinea pig ileum, and evidence has been presented indicating either that PGs enhance and PG synthetase inhibitors reduce ACh release, or that in neither respect there is any effect on the release of ACh (Giblin *et al.* 1973; Botting & Salzman 1974; Kladec *et al.* 1974; Kadlec *et al.* 1974, 1978; Hall *et al.* 1975; Haza 1975; Ehrenpreis *et al.* 1976). In all these studies ACh was measured biologically using the guinea pig ileum as assay tissue. It is obvious that PGs and PG synthetase inhibitors have profound effects on the guinea pig ileum, and it is conceivable therefore that interference with the assay tissue could explain the divergent results in the above-mentioned studies. In order to circumvent this possibly erroneous factor GEMs was used in the present study for identification and quantification of ACh released from the guinea pig ileum and the bovine sphincter.

PGE_2 is tested over a wide range of concentrations and two stimulation frequencies were used (0.5 and 3 Hz) at which PGE_2 is known to have marked effects on induced contraction responses. Although these favourable conditions would seem to be present, we could not detect any effect of PGE_2 on ACh release from the guinea pig ileum or the bovine iris, respectively of whether the preparation is stimulated or not.

It is conceivable that eserine treatment, necessary for preservation of released ACh, may mask an effect of exogenous PGE_2 on transmitter secretion. This eserine markedly raises muscular tone in guinea pig ileum and bovine iris. In the former tissue followed by an increased PG synthesis (Botting & Salzman 1974). Presently it is not known whether the same holds true for the iris, but it is well established that an increase in tone is associated with an increased PG formation also in this tissue (Posner 1973). In both tissues the eserine-induced rise in muscular tone is effectively antagonized by atropine, presumably leading to a decrease in PG formation, as has been demonstrated, e.g. in bronchial tissue (Orehek, Douglas & Bonhays 1975). It was decided therefore to test the effect of PGE_2 on ACh release in the presence of atropine or indomethacin, which also reduces muscular tone and which potently inhibits PG synthesis (Botting & Salzman 1974).

After treatment of the guinea pig ileum and bovine iris sphincter with atropine in a concentration sufficient to abolish the muscular effects of eserine, PGE_2 was still ineffective in altering the

nerve-induced efflux of ACh. Notably atropine per se enhanced nerve-induced release of ACh in guinea pig ileum and bovine iris, indicating that transmitter secretion was not saturated in the presence of eserine. Previously it was shown that atropine increased the release of ACh induced by nerve stimulation or high potassium in the guinea pig ileum but not in the guinea pig heart and bladder or chicken heart (Kilbinger 1977; Sawynok & Jhamandas 1977; Kilbinger & Krieg 1978; Kilbinger & Wagner 1979). These observations were interpreted in terms of some tissues lacking prejunctional muscarinic receptors or a stimulant effect, when seen being due to blockade of inhibitory ganglionic muscarinic receptors (Kilbinger & Krieg 1978; Kilbinger & Wagner 1979). In the present study atropine increased ACh release equally effectively in guinea pig ileum and the ganglion-free bovine iris sphincter. It may be concluded therefore that postganglionic cholinergic nerve terminals seem to possess muscarinic receptors, possibly operated to feed-back control transmitter secretion.

Indomethacin inhibited nerve-induced release of ACh in the guinea pig ileum as observed by others (Kadlec *et al.* 1974; Hall *et al.* 1975). In the presence of indomethacin PGE_2 tended to increase ACh release. Seemingly these observations indicate that PGE_2 may have some stimulant effect on the release process, at least when transmitter secretion is depressed by other agents than ACh itself. Even so the significance of PGE_2 -mediated enhancement of ACh release seems rather unclear when considering the different effect of indomethacin or PGE_2 on ACh release in eserinated and on contraction responses in non-eserinated ileum. Such a comparison is certainly warranted from the point of view that esermination did not alter the efflux of PGE_2 during nerve stimulation of this tissue (Botting & Salzman 1974). Thus the inhibitory effect of indomethacin on ACh release developed very slowly and did not exceed 40% as compared to the rapid and almost complete inhibition of the contraction response. Likewise the enhancement of ACh release by subsequently administered PGE_2 was but small and developed slowly as compared to the rapid and usually complete recovery of contraction responses. It may be concluded therefore that PGE_2 -mediated enhancement of ACh release if at all present, is of considerably less importance than its postfunctional effects.

The present results lend further support to the

concept of an at least in vitro endogenous PG mechanism modulating smooth muscle tone and contraction responses to cholinergic nerve stimuli at the postganglionic postjunctional level. Evidence was not found for PGE_2 being a significant modulator of ACh release. It was also found that atropine increased nerve induced release of ACh in guinea pig ileum and bovine iris in all probability by blocking postganglionic prejunctional muscarinic receptors.

This study was supported by grants from Karolinska Institutet, Stiftelsen Lars Hiertas Minne and the Swedish Medical Research Council (projects 04X-00199 and 04X-4342). The skilful technical assistance of Mr Jan Lundin is gratefully acknowledged.

REFERENCES

- BAUM T & SHROPSHIRE A T 1971 Influence of prostaglandins on autonomic responses. *Am J Physiol* 221 1470-1475.
- BEANI L, BIANCHI C & CREMA A 1969 The effect of catecholamines and sympathetic stimulation on the release of acetylcholine from the guinea-pig colon. *Brit J Pharmacol* 36 1-17.
- BEATTIE J C & STILWELL, D L Jr 1961 Innervation of the eye. *Anat Rec* 141 45-61.
- BENNETT A & POSNER J 1971 Studies on prostaglandin antagonists. *Brit J Pharmacol* 42 584-594.
- BENNETT A, ELEY K G & STOCKLEY H 1975 Modulation by prostaglandins of contractions in guinea-pig ileum. *Prostaglandins* 9: 377-384.
- BERGSTROM S, ELIASSON R, EULER U S v & SJÖVALL J 1959 Some biological effects of two crystalline prostaglandin factors. *Acta Physiol Scand* 45 133-144.
- BOTTING J H & SALZMANN R 1974 The effect of indomethacin on the release of prostaglandin E_2 and acetylcholine from guinea-pig isolated ileum at rest and during field stimulation. *Brit J Pharmacol* 50: 119-124.
- DAVISON P, RAMWELL, P W & WILLIS A L 1972 Inhibition of intestinal tone and prostaglandin synthesis by 5, 8, 11, 14-tetraenoic acid. *Brit J Pharmacol* 46 547P-548P.
- EHRENPREIS S, GREENBERG J & BELMAN S 1973 Prostaglandins reverse inhibition of electrically induced contractions of guinea-pig ileum by morphine. *Indomethacin and acetylsalicylic acid*. *Nature New Biol* 245 280-282.
- EHRENPREIS S, GREENBERG J & COMATY J E 1976 Block of electrically induced contractions of guinea pig longitudinal muscle by prostaglandin synthetase and receptor inhibitors. *Europ J Pharmacol* 39: 331-340.
- FERREIRA S H, HERMAN A & VANE J R 1972 Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum. *Brit J Pharmacol* 44 328-330.
- FERREIRA S H, HERMAN A G & VANE J R 1976 Prostaglandin production by rabbit isolated jejunum and its relationship to the inherent tone of the preparation. *Brit J Pharmacol* 56 469-477.
- FLOWER R J 1974 Drugs which inhibit prostaglandin biosynthesis. *Pharmacol Rev* 26 33-67.
- GUSTAFSSON L, HEDQVIST P & LAGER K 1975 CRANTZ, H 1975 Potentiation by prostaglandins of E_1 , E_2 and $\text{F}_{5\alpha}$ of the contraction response to transmural stimulation in the bovine iris sphincter muscle. *Acta Physiol Scand* 95 76-33.
- GUSTAFSSON L, HEDQVIST P, FREDHOLM B & LUNDGREN G 1978 Inhibition of acetylcholine release in guinea pig ileum by adenosine. *Acta Physiol Scand* 104 469-478.
- HADHÁZY P, ILLES P & KNOLL J 1973 The effects of PGE_2 on responses to cardiac vagus nerve stimulation and acetylcholine release. *Europ J Pharmacol* 23 251-255.
- HALL W J, ONEILL P & SHEEHAN J B 1975 The role of prostaglandins in cholinergic neurotransmission in the guinea pig. *Europ J Pharmacol* 34 79-74.
- HARRY J D 1968 The action of prostaglandin E on the guinea-pig isolated intestine. *Brit J Pharmacol* 33 215P-214P.
- HAZRA J 1975 Evidence against prostaglandin E having a physiological role in acetylcholine liberation from Auerbach's plexus of guinea-pig ileum. *Experientia* 31 565-566.
- HEDQVIST P, GUSTAFSSON L, HJEMDAHL P & SVANBORG K 1980 Aspects of prostaglandin action on autonomic neuroeffector transmission. In *Advances in prostaglandin and thromboxane research* vol 8 pp 145-1248. Raven Press, New York.
- ILLES P, VIZI E S & KNOLL J 1974 Adrenergic neuroeffector junctions sensitive and insensitive to the effect of PGE_2 . *Pol J Pharmacol Pharm* 26 127-136.
- KADLEC O, MASEK K & SEFERNA I 1974 A modulating role of prostaglandins in contractions of the guinea-pig ileum. *Brit J Pharmacol* 51 565-570.
- KADLEC O, MASEK K & SEFERNA I 1978 Modulation by prostaglandins of the release of acetylcholine and noradrenaline in guinea-pig isolated ileum. *J Pharmacol Exp Ther* 205 635-643.
- KARLÉN B, LUNDGREN G, NORDGREN I & HOLMSTEDT B 1974 Ion-pair extraction and gas-phase analysis of acetylcholine and choline. In *Choline and acetylcholine: Handbook of chemical assay methods* (ed I Hazra) pp 163-179. Raven Press, New York.
- KILBINGER, H 1977 Modulation by oxotremorine and atropine of acetylcholine release evoked by electrical stimulation of the myenteric plexus of the guinea-pig ileum. *Naunyn-Schmiedeberg's Arch Pharmacol* 300: 143-151.
- KILBINGER, H & KRIEG C 1978 Effects of atropine on acetylcholine overflow from perfused chicken hearts. *Naunyn-Schmiedeberg's Arch Pharmacol* 302: Suppl R55.
- KILBINGER H & WAGNER, B 1979 The role of presynaptic muscarinic receptors in regulating acetyl-

- cholinergic release from peripheral cholinergic neurones. *Adv Biosc* 18: 347-351.
- OSERIK, J., DOUGLAS, J. S. & BOUHUYS, A. 1975. Contractile responses of the guinea-pig trachea in *u-v* modification by prostaglandin synthetase-inhibiting drugs. *J Pharmacol Exp Ther* 194: 554-564.
- PATON, W. D. M. & VEZI, E. S. 1969. The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Brit J Pharmacol* 35: 19-28.
- POSTEL, J. 1973. Prostaglandin E_2 and the bovine sphincter papillae. *Brit J Pharmacol* 49: 415-427.
- RADMANOVIC, M. Z. 1975. Prostaglandin E_2 and the effect of coxial electrical stimulation of the guinea-pig isolated ileum. *Proc 6th Int Congr Pharmacol, Helsinki* pp 158.
- SACHS, C. & JONSSON, G. 1975. Mechanisms of action of 6-hydroxydopamine. *Biochem Pharmacol* 4: 1-8.
- SAWYNOK, J. & JHAMANDAS, K. 1977. Muscarinic feedback inhibition of acetylcholine release from the myenteric plexus in the guinea pig ileum and its status after chronic exposure to morphine. *Can J Physiol Pharmacol* 55: 909-916.
- SZERB, J. C. 1979. Autoregulation of acetylcholine release. *Adv Biosc* 18: 793-798.

Distensibility of left atrium in normotensive and spontaneously hypertensive rats

S.-E. RICKSTEN, T. YAO, B. LJUNG and P. THORÉN

Department of Physiology, University of Göteborg, Sweden

RICKSTEN S.-E., YAO T., LJUNG B. & THORÉN P. Distensibility of left atrium in normotensive and spontaneously hypertensive rats. *Acta Physiol Scand* 1980; 110: 413-418. Received 13 March 1980. ISSN 0001-6772. Department of Physiology, University of Göteborg, Sweden.

In spontaneously hypertensive rats (SHR) left atrial mechanoreceptors are reset. Thus, left atrial pressure must be almost twice as high in SHR as in normotensive rats to produce comparable degrees of receptor activation and reflex sympathetic inhibition. The present study was performed to investigate whether this resetting is due to decreased atrial distensibility in SHR. Static load-length relationships were therefore investigated on bio-isolated left atrial strips from 11 pairs of male SHR and Wistar Kyoto rats (WKR). After each experiment the strips were fixed at a passive tension of 4 mN and the average wall thickness was determined histologically. Furthermore pressure-volume relationships were studied on non-beating, isolated left atria from SHR and WKR. Distensibility was here defined as % volume increase when LAP was increased from 2.5 to 12.5 mmHg either rapidly (0.5-1 s, dynamic distension) or slowly (3 min, static distension). Atrial wall thickness did not differ significantly in SHR and WKR, but the passive force (mN) per cross-sectional area exerted during elongation above 80% was greater ($P < 0.05$) in SHR. Also the "dynamic" but not the "static" volume distensibility was significantly lower in SHR ($P < 0.01$). The decreased dynamic distensibility of SHR left atrial walls can at least partly explain the resetting of the atrial receptors activated during the rapid filling phase.

Key words. Left atrial mechanoreceptors, resetting, atrial distensibility, SHR.

Cardio-pulmonary receptors are spread throughout the heart, in the atria as well as in the ventricles, and also in the pulmonary veins (cf. Thorén 1979). The majority of these volume receptors fire in myo-electric vagal afferents and activation of receptors induces reflex bradycardia and dilation of resistance and capacitance vessels, e.g. when blood volume is increased. Thorén et al. (1979) found that the cardio-pulmonary receptors were reset in the spontaneously hypertensive rat (SHR) i.e. a great atrial pressure was needed to activate these receptors in SHR (10 mmHg) compared to normotensive controls (~5 mmHg). Ricksten et al. (1979) showed that higher distending pressures in the left heart were needed in SHR to inhibit renal sympathetic nerve activity to the same extent as in normotensive controls. The mechanism behind this resetting of the cardio-pulmonary stretch receptors in the hypertensive animal is not known. Receptor resetting can be due to a change in the receptor

properties per se or/and to a structural change of the left atrial wall. Aars (1969) showed that the resetting of the aortic baroreceptors in renal hypertensive rabbits was caused by a decreased distensibility of the aortic wall and not by a decreased sensitivity of the stretch receptor itself. Other studies, however, indicate that alterations of the receptor properties can contribute to the resetting of the arterial baroreceptors in SHR at least in early stages in hypertension (Andresen et al. 1978). The aim of the present study was to examine the distensibility of the left atrial wall where most of the left cardiac stretch receptors seem to be located in rats (Thorén et al. 1979) further to explore whether decreased wall distensibility could explain the resetting of the left atrial receptors in SHR when compared to normotensive Wistar Kyoto rats (WKR).

On leave from Department of Physiology, Shanghai First Medical College, Shanghai, China.

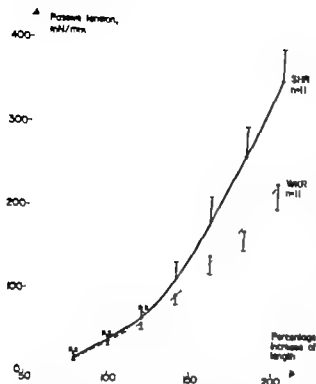


Fig. 1 Static load-length relationship of isolated left atrial strips from SHR and WKR. Only at a relative length increase of more than 80% SHR show a greater passive tension per unit tissue area ($P < 0.05$ $P < 0.01$).

METHODS

Elastic properties of isolated left atrial wall. For this purpose eleven pairs of male SHR weighing 302 ± 3 g and having a mean arterial blood pressure (MAP) of 163 ± 3 mmHg and Wistar Kyoto rats (WKR) with an MAP of 111 ± 3 mmHg and a body weight of 308 ± 3 g, were used. MAP was measured some days before the experimental procedures via a tail artery catheter after recovery from brief ether anaesthesia.

Under ether anaesthesia the heart and lungs were excised from the rat and placed in ice-chilled Krebs solution. After removal of the left auricle a standardized strip was excised from the lateral aspect of the left atrial wall with a width of 0.5–1 mm and a length of 3–4 mm. The strip was tied at both ends with fine silk and mounted vertically between force transducer and a tissue support in an organ bath containing oxygenated Krebs solution at a temperature of 38°C . Throughout the experiment the tissue was activated by electrical field stimulation at 3 Hz with pulses of 4 ms duration at supramaximal intensity. During an initial accommodation period of 60 min the passive force was repeatedly adjusted to 4 mN. At the end of this equilibration period the length of the tissue was determined by means of a Lertz dissection microscope with a micrometer eye piece and the value was defined as the reference length (100%). The strip was then stretched in steps of 0.2 mm at the early flat part of the length-tension curve and 0.1 mm at the steep part of this length-tension curve.

At the end of the experiment the tissue was fixed at passive tension of 4 mN in Bouin's solution overnight. After fixation the preparation was dehydrated in alcohol cleared in xylene, embedded and sectioned perpendicularly to the longitudinal axis of the strip. Sections corresponding to the midpoint along the length of the atrial strip were selected and stained with Weigert's hematoxylin as van Gieson's method. The sections were photographed in a Zeiss photomicroscope at $400\times$ magnification. In all sections the myocardial fibres were cut transversely, i.e. the fibres were orientated in the longitudinal direction of the tissue. The total cross-sectional area of the magnified image of the strip was measured by means of a planimetry method. The width of the tissue was determined at the well defined endocardial aspect of the cross-section. The average thickness of the strip was calculated by dividing the area value with the width of the left atrial strip. The shape of the cross-sections was approximately rectangular.

In each experiment the relationship between the percentage length increase of the strip beyond the reference length (strain) and the passive force per cross-sectional area (stress) was plotted. Due to stress relaxation there was a decay in passive force at each increase in strain and the steady state value was used in the calculations. Reduction of cross-sectional area of the strip during the elongation was not corrected for. The difference between SHR and WKR was compared using Student's *t*-test of pairing design. *P* values below 0.05 were considered significant.

Pressure-volume relationship of the isolated left atrium

16 male SHR and seventeen male WKR weighing 314 ± 5 and 355 ± 4 g respectively were compared in this study. Their MAP values were 157 ± 5 mmHg and 106 ± 2 mmHg respectively. During ether anaesthesia heart and lungs were excised and kept moist by ice-chilled Krebs solution during the preparation and the experimental procedure. The right middle and the left pulmonary veins were cannulated (PE 50) while other veins were ligated close to the respective lung lobes. To prevent communication between the left atrium and ventricle a light ligature was placed around the heart just below the mitral valves. Left atrial pressure (LAP) was recorded via the catheter in the left pulmonary vein with the recording transducer at the level of the left atrium. The isolated preparation thus consisting of the left atrium and adjacent parts of the pulmonary veins was expanded with saline saturated with 2mM Cr EDTA, via the catheter placed in the right middle pulmonary vein. The blood content was first removed out great care being taken to exclude air bubbles. Prior to volume expansion the atrial preparation was allowed to empty passively against atmospheric pressure. No collapse of the atrium was seen during this procedure. From this level left atrial volume was increased stepwise by adding 43 μl of saline via a step injector starting at 300 ms intervals. For each step there was rapid (100–150 ms) increase of LAP which promptly levelled off when the 43 μl had been injected (Fig. 1a, b). In such a way LAP was increased in steps from the basal level of zero mmHg up to around 15 mmHg. Thereafter the atrium was allowed to empty passively to gain reach the basal level. This procedure was repeated 5 times in each experiment and the pressure of

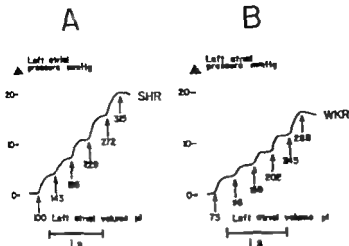


Fig. 2 A typical experiment from SHR and WKR showing recording of atrial pressure and volume from isolated left atrium during rapid distension of the nonbeating atrium.

ness curves proved reproducible in all experiments. At the end of the experiment the absolute atrial volume was estimated as follows. 87 From the basal level 86 μ l of fluid saline was injected into the left atrium. This content was then washed out with ml of normal saline and the effluent was collected for spectrophotometrical determination of the Cr-EDTA concentration ($[Cr-EDTA]$) and for volume measurement (V). The following equation was then used to estimate absolute left atrial volume:

$$\text{Left atrial volume} = \frac{[Cr-EDTA]}{V} \times 86$$

The calculated value of the left atrial volume minus 86 μ l was then the volume at the basal level where LAP was zero.

Three consecutive estimations of left atrial volume were performed in each experiment and the average coefficient of variation was 9–10%. In each experiment the relation between LAP and either absolute or relative volume changes was plotted and average pressure-volume curves were constructed for each group. Distensibility of the left atrial wall was defined as percentage atrial volume increase per mmHg when increasing LAP from 2.5 mmHg to 12.5 mmHg. In all experiments left atrial distensibility was determined by three rapid volume expansions and therefore called 'dynamic distensibility'. In 6–7 experiments of each group the left atrium was also distended slowly from the basal level by stepwise infusions of 39 μ l during 25 s, LAP reaching 15 mmHg in about 3 min. So-called 'static distensibility' was estimated from these values as described above. Also the percentage difference between the dynamic and static distensibility was calculated in each experiment. The difference between SHR and WKR was compared using group comparison *t*-test. *P* values below 0.05 were considered as significant. In the text experimental results are given as mean \pm S.E. unless otherwise noted.

RESULTS

Properties of isolated strips The reference length of the strips in the two groups, i.e. the distance between the ligatures at a passive force of 4 mN was 2.8 ± 0.16 mm for WKR and 3.3 ± 0.18 for SHR respectively. The width of the strips as determined histologically was for WKR 0.548 ± 0.029 mm and for SHR 0.560 ± 0.036 mm. The cross-sectional areas of the dissected left atrial strips were 0.115 ± 0.011 mm² for WKR and 0.117 ± 0.012 mm² for SHR. Thus the dissection procedure yielded comparable tissue segments and there was no significant difference between WKR and SHR concerning the average wall thickness of the atrial strips, which was 0.11 ± 0.019 mm and 0.207 ± 0.012 mm respectively. The results of the studies on the relationship between the relative length (increase of the strip (strain)) and the passive force per cross-sectional area (stress) exerted by the successively extended strip are shown in Fig. 1. Only at a length increase of 80% or more there is a significantly greater passive tension in the SHR tissue compared to WKR. Both in SHR and WKR the maximum active tension is reached at an elongation of the paced strip by 20–30%.

Pressure-volume relationship of the isolated left atrium. A typical pressure-volume recording of the rapidly distended, nonbeating isolated left atrium for SHR and WKR is shown in Fig. 2. Such a recording showed very little variation at multiple runs in each experiment. If there was a continuous de-

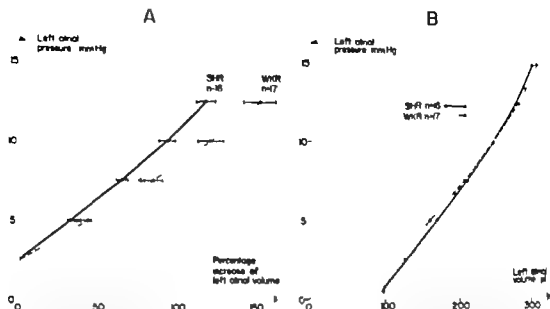


Fig. 3a The average relationship between relative volume (increase of left atrium) and LAP in SHR and WKR during rapid distension of left atrium. The dynamic distensibility is significantly ($P < 0.01$) decreased in SHR.

Fig. 3b The average relationship between absolute left atrial volume and LAP. Error bars are omitted for clarity.

development of rigor or oedema of the left atrial wall during the preparation and the experimental procedure a greater LAP at each left atrial volume would have developed with time. The volume of the isolated left atrium at zero pressure tended to be greater in SHR compared to WKR 0.097 ± 0.005 ml versus 0.079 ± 0.007 ml ($P = 0.053$). The relationship between LAP and relative as well as absolute volume changes of left atrium for the two groups is shown in Fig. 3a, b respectively. A significantly greater relative increase of atrial volume is needed for WKR to achieve the same LAP as in SHR when rapidly increasing the LAP from 2.5 mmHg up to 12.5 mmHg. The values for the dynamic distensibility were 11.7 ± 0.6 mmHg for SHR and 15.7 ± 1.2 mmHg for WKR ($P < 0.01$). Thus the left atrial wall of SHR is about 23% less distensible than that of WKR when increasing LAP rapidly (0.5–1 s) from 2.5 mmHg to 12.5 mmHg.

Fig. 4 shows the average values of the pressure-volume curves for both SHR and WKR from the experiments where the atrium was expanded both rapidly and slowly. When distending the left atrium slowly (3 min) the distensibility of the atrium increased markedly in both groups. The static distensibility was 18.3 ± 1.2 mmHg for SHR and 19.1 ± 1.1 mmHg for WKR ($P = 0.49$). The percent difference between the dynamic and the static distensibility was significantly greater in SHR

compared to WKR $79.8 \pm 3.9\%$ versus $16.9 \pm 1.5\%$ ($P < 0.01$).

DISCUSSION

The cardiac stretch receptors, mainly located in the left atrial wall of the rat, are activated during the rapid filling phase of the heart (Thoren et al. 1978). A greater LAP is needed in SHR (~10 mmHg) to activate these receptors compared to normotensive controls (~5 mmHg). This resetting in SHR is either due to changes of the receptor itself or/and to a decreased distensibility of the left atrium, i.e. a greater LAP is needed in SHR to cause the same distension of the left atrial wall. The present study was designed to elucidate the properties of the left atrial wall, since the properties of the stretch receptor itself cannot be studied directly.

There was no difference in the thickness of the left atrial segments of SHR and WKR when stretched by the same load. These results do not indicate that the left atrial wall of SHR is increased in thickness although the distending LAP is twice as high compared to normotensive control rats (Norell et al. 1979). Greenberg et al. (1978) recently showed signs of hypertrophy in the vascular walls of the caval vein, the portal vein and the pulmonary artery in SHR compared to WKR. However, Mulvany & Ljung (1980) found no difference

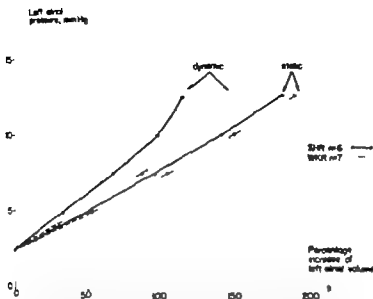


Fig. 4. Average pressure-volume curves from SHR and WKR where LAP is increased either slowly ('static distensibility') or rapidly ('dynamic distensibility') in the same experiment. There was no difference in static distensibility between the two groups. The greatest difference between the dynamic and the static distensibility was significant ($P < 0.01$), by greater in SHR ($P < 0.01$).

in wall thickness of the portal vein when comparing SHR and WKR.

The left atrial strip of SHR developed significantly greater passive tension per unit area compared to WKR only when elongated 80–100% from the reference length. The maximum active stress was reached at an extension of 20–30% from the reference length both in SHR and WKR. A further stretch resulted in a rapid decline in the active tension per unit area. Distension of the strip by 80–100% thus an supraphysiologic overstretch. The greater passive tension at these exaggerated extensions in SHR can perhaps be explained by greater content of collagen fibers per unit area of the strip compared to WKR since these fibres provide the increased stiffness at overstretch at least in arteries (cf. Dobrin 1970).

Aars (1968) studied the elastic properties of aortic strips from renal hypertensive rabbits and found that they were thicker and less distensible per unit cross-sectional area than strips from normotensive rabbits. This decreased elasticity of aortic wall in renal hypertension can thus explain the resetting of the aortic baroreceptors shown by e.g. Aars (1968). In the *in vivo* situation the cardiac stretch recep-

tors are activated during the atrial v wave which lasts for 80–100 ms in the rat (Rucksten, unpublished observation). Thus, the atrial wall is distended very rapidly *in vivo*. Static load-length relationships of left atrial strips or aortic strips do not provide information about their *in vivo* elasticity when subjected to dynamic events such as the rapid filling phase of the heart and arterial pressure oscillations, respectively. It was therefore of particular interest to study the elastic properties of the left atrial wall in SHR during a rapid distension of the left atrium within a physiological pressure range. According to Fig. 2 the time for each injection of 43 μ l saline (100–150 ms) is only slightly longer than the atrial v-wave. It was thus shown that the dynamic distensibility of the left atrium was significantly lower in SHR compared to WKR (Fig. 3a). There was, on the other hand, no significant difference in the static distensibility of the left atrium between the two groups. The decreased dynamic distensibility of the left atrial wall in SHR can in part explain the resetting of the cardiac receptors if it truly reflects distensibility also at the sites of main receptor location. In conscious rats LAP at end expiration is about 10 mmHg and 5 mmHg in SHR and nor-

normotensive control rats respectively (Norellson et al 1979). These values represent the threshold pressure for the left atrial receptor activation in the two groups (Thoren et al 1979) meaning that the receptors become activated in SHR and the normotensive control first at 10 mmHg and 5 mmHg respectively. Assuming that there is no difference between the two groups concerning the characteristics of the receptor itself this means an equal degree of stretch for the SHR atrial receptors at 10 mmHg as for the WKR receptors at 5 mmHg. In other words at the threshold pressure for receptor activation the absolute left atrial volume is equal in the two groups. However in Fig. 3b the absolute values for the left atrial volume is plotted against LAP for the two groups at rapid atrial distension. If this dynamic pressure-volume curve truly mirrors the in vivo situation left atrial volume is about 50% greater in SHR at 10 mmHg compared to WKR at 5 mmHg. Thus other factors than pure distensibility changes is presumably at hand in SHR in order to explain why the receptors start to fire at a LAP of 10 mmHg when left atrial volume is about 50% greater compared to normotensive controls.

In conclusion the dynamic distensibility of left atrial wall is significantly decreased in SHR compared to WKR. This difference can at least partly account for the resetting of the left atrial stretch receptors in SHR.

The expenses for this study were in part covered by grants from the Swedish Medical Research Council (00016 and 04764) from the Medical Faculty University of Göteborg from Svenska Sällskapet för Medicinsk Forskning and from Magnus Bergvalls Stiftelse.

REFERENCES

- AARS H 1968. Static load-length characteristics of the strips from hypertensive rabbits. *Acta Phys Scand* 73 101-110.
- AARS H 1969. Relationship between aortic diameter and aortic baroreceptor activity in normal and hypertensive rabbits. *Acta Physiol Scand* 75 406-414.
- ANDRESEN M C, KRAUTHS J M & BROWN A M 1978. Relationship of aortic wall and baroreceptor properties during development in normotensive and spontaneously hypertensive rats. *Circulat Res* 43 728-738.
- DOBRIN P B 1978. Mechanical properties of arteries. *Physiol Rev* 58 397-461.
- GREENBERG S, PALMER E C & WILBORN W M 1978. Pressure independent hypertrophy of venous and pulmonary arteries of spontaneously hypertensive rats. Characterization of function, structural and biochemical changes. *Clin Sci Mol Med* 55 31-36.
- MULVANY M J, LJUNG B, STOLTZE M & KJELLSTEDT A 1980. Contractile and morphological properties of the portal vein in spontaneously hypertensive and in Wistar Kyoto rats. *Blood Vessels*. To be published.
- RICKSTEN S E, NORELLSON E & THORÉN P 1979. Inhibition of renal sympathetic nerve traffic from cardiac receptors in normotensive and spontaneously hypertensive rats. *Acta Physiol Scand* 106 17-24.
- THORÉN P 1979. Role of vagal C-fibres in cardiovascular control. *Rev Physiol Biochem Pharmacol* 86 1-94.
- THORÉN P, NORELLSON E & RICKSTEN S E 1979a. Resetting of cardiac C fibre endings in the spontaneously hypertensive rat. *Acta Physiol Scand* 107 13-18.
- THORÉN P, NORELLSON E & RICKSTEN S E 1979b. Cardiac receptors with vagal afferents in the rat. *Acta Physiol Scand* 105 293-303.

Stimulation of gastric acid and bicarbonate secretions by calcium in guinea pig stomach and amphibian isolated mucosa

JUNNAR FLEMSSTRÖM and ANDREW GARNER

Department of Physiology and Medical Biophysics, University of Uppsala Biomedical Center, Sweden

FLEMSSTRÖM J. & GARNER, A. Stimulation of gastric acid and bicarbonate secretions by calcium in guinea pig stomach and amphibian isolated mucosa. *Acta Physiol Scand* 1980; 110: 419-426. Received 24 March 1980. ISSN 0001-6772. Department of Physiology and Medical Biophysics, University of Uppsala Biomedical Center, Sweden.

Administration of Ca^{++} (1.5 mg/kg i.v.) increased the output of both H^+ and HCO_3^- from the stomach of the anesthetized guinea pig as determined by measurement of gastric intraluminal pH and pCO_2 . The rise in HCO_3^- secretion was slightly greater than that in H^+ resulting in a decrease in net acidity. Fundic mucosa isolated from frogs was used to study the mechanisms of the stimulatory actions. An increase in Ca^{++} concentration in the nutrient (serosal) bathing solution from 1.8 to 7.2 mM stimulated H^+ transport in this preparation. The effect of raising Ca^{++} concentration was inhibited by the histamine H_2 receptor antagonist Metamizol and by increasing nutrient Mg^{++} . Stimulation of H^+ transport, sensitive to Metamizol, was also observed with the calcium ionophore A23187 (4 $\mu\text{g/ml}$ nutrient side). The results indicate that at the mucosal level, Ca^{++} stimulates H^+ transport by release of histamine from mucosal stores with properties similar to those of most cells. Transport of HCO_3^- in isolated mucosae was studied after inhibition of H^+ transport by metamizol. An increase in nutrient Ca^{++} concentration stimulated the HCO_3^- transport but the calcium ionophore had no effect. This action of Ca^{++} was abolished by atropine (10^{-6} M) and by raising nutrient Mg^{++} suggesting that it reflects release of acetylcholine from intramucosal nervous tissue. Thus Ca^{++} stimulated gastric transport of both H^+ and HCO_3^- in vivo and in vitro but evidence for a direct action on the transporting (parietal and epithelial) cells was not obtained.

Key words. Calcium, calcium ionophore, magnesium, gastric H^+ secretion, gastric HCO_3^- secretion

Recent studies in vitro have shown that antral and fundic mucosae obtained from a variety of amphibians actively transport HCO_3^- into the gastric lumen (Flemström & Sachs 1975; Flemström 1976, 1977). Secretion of HCO_3^- has also been demonstrated in the guinea pig stomach and dog fundic pouch in vivo (Garner & Flemström 1978; Kauffman, Reeve & Grossman 1980). It has been proposed that the HCO_3^- , probably originating from surface epithelial cells, protects the mucosal surface from intraluminal acid (cf. Flemström 1978). Inhibition of HCO_3^- transport by a variety of ulcerogenic agents, including acetazolamide, alpha-adrenergic agonists and nonsteroidal anti-inflammatory drugs as well as stimulation of secretion by gastric ulcer-protective prostaglandins support this concept (Garner 1977, 1978; Flemström 1981; Garner, Flemström & Heylings 1979).

Gastric transport of HCO_3^- is usually smaller and thus masked by the simultaneous secretion of acid but can be revealed by specific inhibition of the latter (Flemström 1977). Previous studies on the effect of calcium ions on gastric electrolyte transport have considered the combined secretions of H^+ and HCO_3^- , i.e. the net acid output. Depending on species and experimental conditions, administration of Ca^{++} has been found to either increase or decrease net acidity although the recorded effects are often relatively small (Barreras 1973). A Ca^{++} induced rise in HCO_3^- transport has been observed previously in isolated amphibian antrum (Flemström 1978). It was therefore of interest to compare the effects of Ca^{++} administration on gastric tran-

Present address: Department of Biology, ICI Pharmaceuticals, Macclesfield, England

sport of both H⁺ and HCO₃⁻. It was found in the guinea pig stomach that a greater rise in HCO₃⁻ transport masked a simultaneous rise in H⁺ transport. Isolated fundic mucosae were used for further study of the stimulatory mechanisms.

METHODS AND MATERIALS

In vivo experiments

A full description of the method has been published recently (Garner & Flemström 1978). Male albino guinea pigs (380–420 g) were starved for 24 h in cages with mesh bottoms to minimize coprophagy but allowed free access to drinking water. Animals were anaesthetised with urethane (1.5 g/kg intraperitoneally). Body temperature was maintained at 37°C with a heated blanket controlled by an intrarectal thermistor. The abdomen was opened by a median epigastric incision and a multi-orifice rubber tube passed into the stomach through a cut in the duodenal wall proximal to the insertion of the bile duct. The tube was secured in place by a ligature around the pylorus and exteriorised via the right flank. The trachea was cannulated. An umbilical artery catheter (8F Sherwood Medical Industries, St. Louis, USA) was passed down the oesophagus into the stomach so that the tip terminated just beyond the cardia. The catheter was held in place by a tie around the oesophagus but excluding the agli which also prevented contamination of gastric samples by salivary secretion. The stomach was rinsed clear of food debris. Then 10 ml volumes of mannitol solution (310 mM) were instilled into the stomach via the oesophageal catheter and removed through the duodenal tube every 10 min.

Intragastric pCO₂ was determined at the end of each 10 min instillation period by withdrawing samples from the stomach through glass capillary tubing into a pCO₂ electrode (Radiometer E5036). Intragastric pH was measured by a glass calomel electrode (Radiometer OH-321). Both electrodes were connected to expanded scale pH-mV meters (Radiometer PHM26).

Total H⁺ and HCO₃⁻ in each instillate was calculated as described before (Garner & Flemström 1978): total HCO₃⁻ as free HCO₃⁻ (calculated from the pCO₂ and pH values) plus dissolved CO₂ formed during reaction between secreted HCO₃⁻ and H⁺. Concentrations of Na⁺ and K⁺ in the gastric instillates were determined by flame photometry (Eppendorf, Neethler and Hinz, Hamburg) and Cl⁻ was determined by titration (Radiometer CMT 10).

In vitro experiments

The method has previously been described in detail (Flemström & Sachs 1974; Flemström 1977). Frogs (*Rana temporaria*) were purchased from Firma Panzer, Laingingen-Donau, FRG. The animals were kept in tap water at 10°C for up to two months and force fed with ~200 mg of liver once a week. The fundic mucosa (and submucosa) was separated from the serosa and muscle layers by blunt dissection in an unbuffered solution and mounted as a membrane between the two halves of an in vitro chamber. The exposed mucosal area was 1.8 cm².

For comparison, antral mucosa was used in some experiments. Antrum of sufficient size was obtained from *Vecturus* or *Rana catesbeiana* and distinguished for fundus by its paler appearance.

Each side of the mucosa was bathed with 30 ml of solution circulated by a gas lift. An unbuffered solution gassed with 100% O₂ prewashed in Ba(OH)₂ to exclude possible traces of CO₂, was used on the luminal (secretory) side. The pH of this fluid was kept constant at 7.4 by infusion of iso-osmolar solutions containing 1 mM HCl or 10 mM NaOH under automatic control from a pH-stat system (Titrator TTT[®] and Autoburette ABU 13, Radiometer, Copenhagen). The amount of base or acid transported into the luminal solution was calculated from the amount of HCl or NaOH infused. The nutrient (serosal) solution (pH 7.20) was buffered with HCO₃⁻ (17.8 mM) and phosphate (0.8 mM) and gassed with O₂/CO₂ (95/5 vol./vol.). The standard luminal and nutrient solutions contained (in mM): Na⁺ 102.4, K⁺ 4.0, Ca²⁺ 1.8, Mg²⁺ 0.8 and Cl⁻ 91.6; mannitol and SO₄²⁻ being used on the luminal side to achieve iso-osmolality (221 mosM). Open circuit transmucosal electrical potential difference was measured via matched calomel electrode and recorded on a high-input impedance voltmeter. Electrical resistance was determined from the immediate (~1 sec) change in potential difference after steady current (30 µA/cm²) through the mucosa in either direction. Experiments were performed at 20°C.

Drugs were administered on the nutrient side of the mucosa only. Metiamide and calcium ionophore A23187 were kindly donated by Smith, Kline and French Laboratories, Welwyn Garden City, England and E. Lilly Sweden AB, Stockholm. Atropine (as sulphate) was obtained from Sigma, St. Louis, USA. To examine the effect of raised Ca²⁺ or Mg²⁺ concentrations, the regular nutrient solution was replaced with solutions containing 7.2 mM Ca²⁺ or Mg²⁺ in which osmolality was made lower by adjusting Na⁺ concentration. Concentrations of Ca²⁺ and Mg²⁺ on the luminal side were always 1.8 and 0.8 mM, respectively.

Statistical analyses

Secretory rate and electrical properties *in vitro* were recorded every 5 min and mean values for consecutive 15 min periods calculated for individual experiments. Overall mean \pm S.E. of mean for each experimental group is presented. Ion outputs *in vivo* were determined every 10 min. Statistical significance in all experiments was determined using Student's *t* test for paired variables by comparing data during the 30 min period before exposure to Ca²⁺, Mg²⁺ or ionophore with 30 (or 15 min) data during and after administration.

RESULTS

Stability conditions in controls

Mean rates of alkaline secretion in the guinea pig stomach *in vivo* and in Metiamide-treated fundus (*Rana temporaria*) *in vitro* are shown in Fig. 1. Secretory rate was stable in the guinea pig whereas

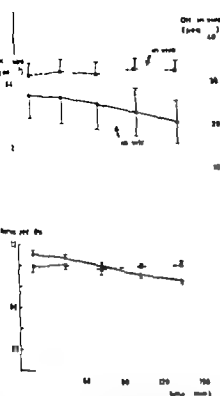


Fig. 1 Control rates of gastric alkaline secretion in frog and guinea pig stomach in vivo. Rates of secretion during consecutive 30 min periods are calculated for each preparation. Overall mean \pm S.E. at each time point is shown ($n=6$ for each group). Although there was considerable variation in secretory rate between individual preparations, some experimental error was minimal as shown by the normalized data.

secretion in vitro showed slow but continuous decline. In both preparations, S.E. of the mean was large. However this was not due to variation within experiments but as the result of differences in secretory rate between individual preparations as shown by the normalized values. These were obtained by division of the rate in each 30 min period by the mean rate for all consecutive 30 min periods in the same experiment. Thus in studying the effects of ions or drugs on HCO_3^- transport, it was advantageous to use each experiment as its own control.

Gastric H and HCO_3^- transport in the guinea pig

The effects of 1 injection of 1.5 mg/kg elemental Ca^{2+} (as chloride) is shown in Fig. 2. Both HCO_3^-

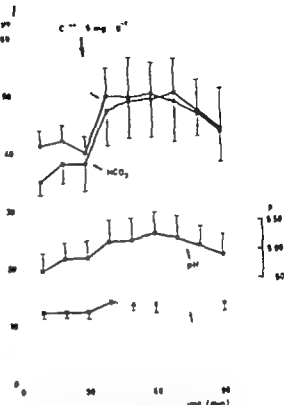


Fig. 2 In the guinea pig in vivo injection of Ca (1.5 mg/kg intravenously) stimulated secretion of both H and HCO_3^- and also increased K output. Although peak H output occurred earlier HCO_3^- secretion rose by more than H secretion and rise in pH occurred. Mean \pm S.E. is shown ($n=6$).

and H secretion increased significantly ($0.01 < P < 0.05$ for HCO_3^- and $0.02 < P < 0.05$ for H) during the 30 min period after injection. Secretion of HCO_3^- was stimulated somewhat more than H as indicated also by the rise ($0.01 < P < 0.02$) in pH although peak H output appeared more rapidly in most of these expts. There was also a significant rise in K output ($0.02 < P < 0.05$). Output of Cl $^-$ increased from 153 ± 9 to 159 ± 11 and Na from 134 ± 15 to 141 ± 11 $\mu\text{eq h}^{-1}$ but these changes were not significant ($P > 0.05$).

The rise in HCO_3^- output in the guinea pig is in line with the previous finding (Flemström 1978) of stimulation of HCO_3^- transport by Ca in the isolated amphibian antrum. Relations between serum Ca levels, calcemic hormones and gastrointestinal function is, however, complex (Cooper

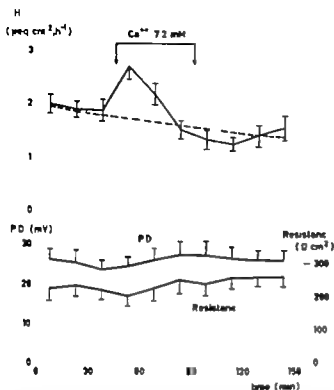


Fig. 3 Effect of increasing Ca^{2+} concentration in the nutrient (serosal) bathing fluid from 1.8 to 7.2 mM on titratable H^{+} secretion and electrical properties of isolated fundic mucosa. Mean \pm S.E. is shown ($n=10$). Spontaneous H^{+} secretion in an untreated preparation (dashed line) is also shown.

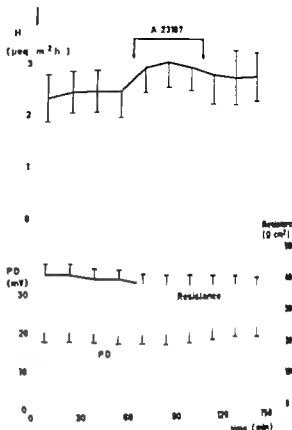


Fig. 4 The calcium ionophore A23187 ($4 \mu\text{g/ml}$ nutrient side) caused a significant increase in the rate of spontaneous net H^{+} secretion in isolated mucosa but did not affect the electrical potential difference or resistance. Mean \pm S.E. is shown ($n=8$).

et al 1978). Gastric H^{+} transport is under hormonal influence and evidence obtained in the dog suggests that this is also the case for gastric HCO_3^- transport (cf. Allen & Garner 1980). Isolated mucosae were therefore chosen in the present study for further evaluation of the stimulatory actions of Ca^{2+} on gastric ion transport. Amphibian preparations were employed since it seems difficult to adequately oxygenate mammalian preparations at atmospheric oxygen pressure (Davenport & Jensen Chavré 1950; Flemström 1971).

H^{+} secreting isolated mucosa

Spontaneous (net H^{+}) secreting fundic mucosae were obtained from *Rana temporaria*. Elevation of nutrient Ca^{2+} from 1.8 to 7.2 mM increased H^{+} secretion ($P<0.01$) but the rise was of only 30 min duration (Fig. 3). Secretion then decreased to values below those expected from the spontaneous decline occurring in untreated mucosae and after return to 1.8 mM nutrient side Ca^{2+} there was some recovery in secretory rate. There was thus a

two-phase response to raising Ca^{2+} concentration: an increase in net H^{+} secretory rate followed by a decrease. Only minor changes in the electrical potential difference and resistance occurred during these experiments.

The calcium ionophore A23187 ($4 \mu\text{g ml}^{-1}$) increased the rate of (net) H^{+} secretion ($P<0.01$) but produced no changes in the electrical parameters of the mucosa (Fig. 4). A lower concentration of the ionophore ($0.8 \mu\text{g ml}^{-1}$, $n=6$) had no significant effects. The ionophore was added as a small volume from an ethanol-containing stock solution but the ethanol alone (0.4% vol/vol nutrient side) did not affect H^{+} secretion ($n=7$).

Mg^{2+} antagonizes stimulation of gastric (net) H^{+} secretion by intravenous or intragastric Ca^{2+} in man (Deyhle, Miederer & Ottenjahn 1970; Christiansen, Rehfeld & Kirkegaard 1979). Elevation of Mg^{2+} concentration in the nutrient solution from 0.8 to 7.2 mM (Fig. 5) depressed spontaneous H^{+} secretion and markedly reduced the stimulatory effect of Ca^{2+} (cf. Fig. 3 and 5).

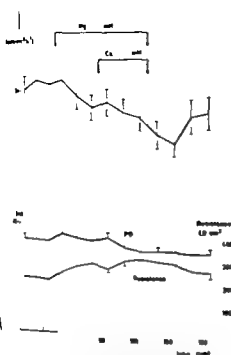


Fig 5 In spontaneously (net H^+) secreting isolated mucosa, antral side Mg^{++} and Ca^{++} concentrations were increased from 1.8 to 7.2 mM as indicated. Mg^{++} inhibited mucosa and the stimulatory effect of raised Ca^{++} . Mean \pm S.E. shown (7).

ACU; secreting isolated mucosa

Fundic mucosae were pretreated with the histamine H_2 receptor antagonist Metiamide (10^{-6} M, mucosal side) and, after net H^+ secretion had ceased and a steady rate of titratable alkalization had appeared, antral Ca^{++} was raised to 7.2 mM (Fig. 6). Secretory rate was slightly but not significantly ($P>0.05$) lower during the first 15 min period but then increased and was significantly ($P<0.01$) higher than the control rate during the final 15 min period of exposure to high Ca . There was an initial rise in the transepithelial electrical potential difference ($0.01<P<0.02$) but no significant change in the electrical resistance.

Fig 6 also illustrates the effects of treatment with atropine (10^{-6} M) or a high Mg^{++} (7.2 mM) concentration. These conditions per se did not affect the rate of alkaline secretion or the electrical parameters ($n=8$ for both) but abolished the stimulatory effect of increased Ca^{++} .

When calcium ionophore A23187 was added to Metiamide treated alkaline secreting mucosae

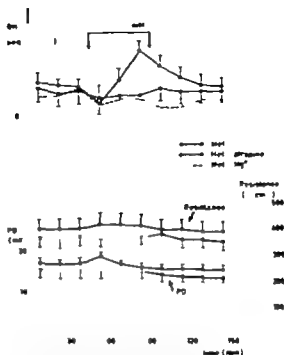


Fig 6 Fundic mucosa was pretreated with Metiamide (10^{-6} M, mucosal side) until net acid secretion had ceased and a steady rate of net alkaline secretion appeared. This drug was then present throughout the experiments. An increase in antral Ca^{++} from 1.8 to 7.2 mM caused slight initial decrease in secretory rate followed by an increase to level above the starting rate ($n=12$). Ca had no effect when atropine (10^{-6} M, mucosal side) was present ($n=9$) or when antral Mg^{++} had been decreased from 0.8 to 7.2 mM ($n=8$, dashed line).

there was no change in the secretory rate even at a concentration of 10 μ g/ml (Fig. 7). Stimulation of H^+ transport (cf Fig. 4) would have resulted in a decline in titratable alkalization and stimulation of HCO_3^- transport in an increase. To exclude the possibility that stimulation of equal magnitude in the rates of H^+ and HCO_3^- secretion occurs some experiments were performed with spontaneously alkalinizing antral mucosae. No effects with the ionophore (4 μ g/ml) were observed in antrum from either *Rana cat belana* ($n=4$) or *Necturus* ($n=5$).

DISCUSSION

Studies in vitro and in vivo have demonstrated that gastric mucosa transports base as well as acid into the lumen of the stomach and that the pre

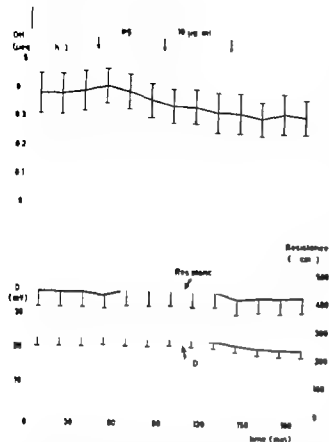


Fig. 7. In isolated fundic mucosa pretreated with Metiamide (10^{-3} M, nutrient side) the calcium ionophore A23187 had no effect on alkaline secretion or electrical properties. There was a slight decline in secretory rate but this was similar to controls (see Fig. 1). Mean \pm S.E. is shown ($n=10$).

vailing net acidity of this combined secretion is the difference between the larger output of H^+ ions and the smaller and therefore masked output of HCO_3^- (Flemström 1977; Garner & Flemström 1978; Kauffman et al. 1980). It is well established that H^+ ions are secreted by parietal cells in the fundic area while *in vitro* studies indicate that HCO_3^- very probably originates from surface epithelial cells in both fundus and antrum (Flemström 1977).

Previous work on the effect of Ca^{2+} on gastric secretion of electrolytes has considered effects on the combined secretions of H^+ and HCO_3^- . Administration of Ca^{2+} has been reported to decrease net acid secretion in the dog and rat *in vivo* (Barreras 1973) and in the amphibian gastric mucosa *in vitro* (Kasbekar & Chugani 1976). In other species including man, ferret, cat and monkey, net H^+ secretion is increased by intra-

venous or intragastric Ca^{2+} (Barreras 1973; Christiansen et al. 1979). The decrease in net acid output observed in some species *in vivo* may only be apparent and reflect greater stimulation of HCO_3^- transport as observed in the present study in the guinea pig (Fig. 7). Raising nutrient Ca^{2+} concentration increased net H^+ secretion *in vitro* (Fig. 3) but this effect was transient, followed by a decline in titratable acidity. This decline in view of results in the isolated antrum (Flemström 1978) and in Metiamide-treated fundic mucosa (Fig. 6) is probably due to an increase in HCO_3^- transport rather than actual inhibition of H^+ transport.

Calcium is important in maintaining normal permeability characteristics of most epithelia. Removal of this ion increases paracellular permeation of ions across the gastric mucosa (Forte & Nauss 1963; Sedar & Forte 1964; Chung et al. 1970). However, transmucosal electrical resistance never decreased as a result of the alterations in nutrient Ca^{2+} employed in the present study. It is thus unlikely that any increase in mucosal permeability to HCO_3^- (or H^+) occurred. Passive permeation of HCO_3^- contributes to luminal alkalinization in antrum (Flemström & Sachs 1975) but not in fundus (Flemström 1977). An increase in paracellular permeation of ions masked by a concomitant decrease of equal magnitude in transcellular ion permeability cannot be excluded but would seem unlikely.

Several mechanisms have been suggested to account for the stimulation of (net) H^+ secretion by Ca^{2+} observed in some species. In man, gastrin released from the antrum or extragastric source contributes to the rise in acid output after administration of Ca^{2+} (Barreras 1973; Christiansen et al. 1979). However, Mg²⁺ antagonises the effect of Ca^{2+} on H^+ secretion in man and under some conditions intragastric Ca^{2+} increases H^+ secretion but not serum gastrin levels (Holttermüller et al. 1974). Thus direct stimulation of the parietal cells by Ca^{2+} or its sensitization to other stimuli has also been postulated.

In the present study, administration of Ca^{2+} increased H^+ secretion *in vivo* and in the fundus *in vitro*. The latter preparation would seem to exclude an effect mediated by release of gastrin. Administration of the calcium ionophore A23187 also increased (net) H^+ secretion *in vitro*. In alkaline-secreting mucosae treated with the histamine H_2 receptor antagonist Metiamide, any stimulation of

H⁺ transport by Ca⁺⁺ and A-3187 comparable in size to that occurring in untreated mucosae would have resulted in a sharp decline in titratable acidity. This is never observed (Figs. 6 and 7), suggesting that stimulation of H⁺ secretion by both Ca⁺⁺ and calcium ionophore is mediated by histamine. As is seen (Deyhle et al. 1970; Christiansen et al. 1979), Mg⁺⁺ depressed both spontaneous H⁺ secretion and the stimulatory effect of increased Ca⁺⁺. Competition between Ca⁺⁺ and Mg⁺⁺ at the release of histamine has been demonstrated in isolated mast cells (Foreman, Mongar & Gomperts 1973) and these cells are proposed to be the major repository for gastric mucosal histamine (Bell, Lewis & Beaven 1979). Furthermore spontaneous H⁺ secretion in isolated mucosae is probably accounted for by histamine released from endogenous sources (Kasbekar 1967; Rangachari 1975).

Previous work has demonstrated that gastric HCO₃⁻ transport is stimulated by carbachol, dibutyryl-GMP and ulceroprotective prostaglandins while histamine and gastrin are without effect. Atropine prevents stimulation of HCO₃⁻ secretion by carbachol in the guinea pig but does not affect basal (unstimulated) HCO₃⁻ transport (Garner & Flenström 1978). It was found in the present study that atropine is also without effect on basal HCO₃⁻ transport in vitro. An increase in external Ca⁺⁺ concentration but not administration of calcium ionophore stimulated HCO₃⁻ transport. The effect of Ca⁺⁺ was abolished by pretreatment with either atropine or increased Mg⁺⁺ concentration. These results suggest that Ca⁺⁺ induced stimulation of HCO₃⁻ transport is mediated by acetylcholine. Release of acetylcholine from nervous tissue in isolated ileal preparations depends on external Ca⁺⁺ and is reduced by external Mg⁺⁺ (Paton & Vizi 1968; Paton, Vizi & Carr 1971). Evidence for a direct effect of Ca⁺⁺ ions on the HCO₃⁻ transporting cell was not obtained in the present study. Thus Ca⁺⁺ stimulates gastric transport of both H⁺ and HCO₃⁻ but effects at the mucosal level are probably mediated by release of histamine and acetylcholine respectively.

REFERENCES

- ALLEN, A. & GARNER, A. 1980. Mucus and bicarbonate secretion in the stomach and their role in mucosal protection. *Gut* 21: 249-262.
- BARRERAS, R. F. 1973. Calcium and gastric secretion. *Gastroenterology* 64: 1168-1184.
- CHRISTIANSEN, J., REHFELD, J. F. & KIRKEGAARD, P. 1979. Interaction of calcium, magnesium and gastrin on gastric acid secretion. *Gastroenterology* 76: 67-61.
- CHUNG, R. S. K., SUM, S. B., GOLDMAN, H., FIELD, M. & SILEN, W. 1970. Effects of the lumen of calcium on the gastric mucosal barrier. *Gastroenterology* 59: 200-207.
- COOPER, C. W., BOLMAN, R. H., LINEHAM, W. M. & WELLS, S. A. 1978. Interrelationships between calcium, calcemic hormones and gastrointestinal hormones. *Recent Prog. Horm. Res.* 34: 259-283.
- DAVENPORT, H. W. & JENSEN-CHAVRÉ, V. 1940. Conditions affecting acid secretion by mouse stomach in vitro. *Gastroenterology* 15: 467-480.
- DEYHLE, P., MIEDERER, S. E. & OTTENJAHN, R. 1970. Der Einfluss akuter Hypercalcämie auf die durch Hypermagnesiämie gehemmte Magensekretion. *Klin. Wochschr.* 48: 384-385.
- FLENSTRÖM, G. 1971. Na⁺ transport and impedance properties of the isolated frog gastric mucosa at different O₂ tensions. *Biochim. Biophys. Acta* 225: 33-43.
- FLENSTRÖM, G. 1976. Properties of isolated gastric antral and SCN⁻ inhibited fundic mucosa. In: *Gastric hydrogen ion secretion* (ed. D. K. Kasbekar, G. Sachs & W. S. Rehm), pp. 102-116. Dekker, New York.
- FLENSTRÖM, G. 1977. Active alkalization by amphibian gastric fundic mucosa in vitro. *Amer. J. Physiol.* 233: E1-E12.
- FLENSTRÖM, G. 1978. Effect of catecholamines, Ca⁺⁺ and gastrin on gastric HCO₃⁻ secretion. *Acta Physiol. Scand., Suppl. Gastric ion transport*, 81-90.
- FLENSTRÖM, G. 1981. Gastric secretion of bicarbonate. In: *Physiology of the digestive tract* (ed. R. L. Johnson, J. Christensen, M. J. Grossman, E. E. Jacobson & S. G. Schultz), in press. Raven Press, New York.
- FLENSTRÖM, G. & Sachs, G. 1975. Ion transport by amphibian antrum in vitro. I. General characteristics. *Amer. J. Physiol.* 228: 1183-1194.
- FOREMAN, J. C., MONGAR, J. L. & GOMPERTS, B. D. 1973. Calcium ionophores and movement of calcium ions following the physiological stimulus to a secretory process. *Nature (Lond.)* 245: 249-251.
- FORTE, J. G. & NAUSS, A. H. 1963. Effects of calcium removal on building gastric mucosa. *Amer. J. Physiol.* 205: 631-637.
- GARNER, A. 1977. Effects of acetylcholinesterase on alkalization, acid secretion and electrogenic properties in the isolated gastric mucosa. *Acta Physiol. Scand.* 99: 281-291.
- GARNER, A. 1978. Mechanisms of action of aspirin on the gastric mucosa of the guinea pig. *Acta Physiol. Scand., Suppl. Gastric ion transport*, 101-110.

I wish to thank Mrs. Carla Fleeth for excellent technical assistance. The study was supported by grants from the Swedish Medical Research Council (04X-3513) and the Royal Society (London).

- GARNER A & FLEMSTRÖM G 1978 Gastric HCO_3^- secretion in the guinea pig. *Amer J Physiol* 234 E535-E541.
- GARNER A, FLEMSTRÖM G & HEYLINGS J R 1979 Effects of anti-inflammatory agents and prostaglandins on acid and bicarbonate secretions in amphibian isolated gastric mucosa. *Gastroenterology* 77 451-457.
- HOLTERMÜLLER, K H, GOLDSMITH R S, SIZEMORE D W & GO V L. W 1974 Dissociation of gastric acid and serum gastrin responses to intraluminal calcium in man: influence of calcitonin and parathyroid hormone. *Gastroenterology* 67 1101-1106.
- KASBEKAR D K 1967 Studies of resting isolated frog gastric mucosa. *Proc Soc Exp Biol Med* 125 267-271.
- KASBEKAR D K & CHUGANI H 1976 Role of calcium in vitro gastric acid secretion. In: *Gastric hydrogen ion secretion* (ed D K. Kasbekar & Sachs & W S. Rehm) pp 187-211. Dekker New York.
- KAUFFMAN G L, REEVE, J J & GROSSMAN M I 1980. Gastric bicarbonate secretion: effect of topical and intravenous 16-16 dimethyl prostaglandin E_2 . *Amer J Physiol* 239: In press.
- PATON W D M & VIZI E. S 1966. The output of acetylcholine released from guinea pig intestine and longitudinal muscle strip. *J Physiol (Lond)* 15 13-33.
- PATON W D M, VIZI E. S. & ZAR, M ABO 1971 The mechanism of acetylcholine release by parasympathetic nerves. *J Physiol (Lond)* 215 81-848.
- RANGACHARI P K 1975 Histamine release by gastric stimulants. *Nature (Lond.)* 253 53-55.
- SEDAR A. W & FORTE, J D 1964 Effects of calcium depletion on the junctional complex between oxyntic cells of gastric glands. *J Cell Biol* 22, 173-188.
- SOLL, A H, LEWIN N & BEAVEN M. 1979 Isolation of histamine-containing cells from canine fundic mucosa. *Gastroenterology* 77 122-1290.

Factor in human CSF with apparent morphine-antagonistic properties

A WAHLSTRÖM and L. TERENIUS

Department of Pharmacology, University of Uppsala, Sweden

Chronic use of opiates leads to development of tolerance. This is a very characteristic phenomenon. The mechanisms that could explain development of tolerance are 1) that the receptor changes in responsiveness on chronic exposure or 2) that counteracting neuronal or endocrine systems, anti-opioids, are activated. The latter possibility has been investigated by us (Terenius 1975), Ungar and co-workers (Ungar & Ungar 1976, Ungar et al 1977) and more recently by Han and co-workers (Han et al 1979). All these investigators obtained evidence for anti-opioid substances believed to act by competition at the level of opiate receptors. However the studied fractions were not well defined chemically and the results are not easy to interpret.

We previously used the human cerebrospinal fluid (CSF) as a source of neuroactive substances, particularly endorphins. We now report on observations in relation to the search for antiopioid substances in CSF of patients, particularly of narcotic addicts.

The lumbar CSF samples were obtained from heroin addicts who had been admitted to hospital for detoxification and start on methadone maintenance therapy. Control CSF was obtained from patients undergoing neurosurgery but who had no history of psychiatric or neurologic disturbances. All samples of CSF were kept frozen at -90°C until analysed. After thawing they were desalted on a RP Sephadex ion-exchanger (15 \times 1 cm) and active material was eluted in 0.5 M pyridine acetate buffer. Further fractionation was accomplished using a Biogel P2 column (160 \times 1 cm) eluted in 0.1% trifluoroacetic acid. This column had previously been calibrated with peptides of known molecular weight. Details of the procedure are published

elsewhere (Wahlström & Terenius 1980). Fractions were lyophilized and tested for activity using either the electrically stimulated myenteric plexus-longitudinal muscle preparation of guinea-pig ileum or the receptor binding assay. When the guinea-pig ileum assay (Paton 1957) was employed fractions were tested either alone or in the presence of normorphine at a dose which reduced the twitch approximately by half (about 10^{-7} M concentration). The receptor binding assay followed a previously described procedure (Terenius 1974): D-Ala²-Leu⁵-enkephalin-H was the radioligand and the fractions were added in graded amounts. Activity was read against a standard curve for D-Ala²-Leu⁵-enkephalin.

Fractionation of material on the Biogel P2 column resolved several components active on the ileum preparation. One fraction with apparent molecular weight, 1000-1500 dalton, suppressed the twitch, i.e. mimicked the action of normorphine. Another fraction of 1500-3000 dalton molecular weight counteracted normorphine (Fig. 1a) whereas in the

Table 1. Relative amounts of a fraction from human CSF potentiating the twitch of the electrically stimulated isolated guinea-pig ileum

Origin of CSF	Pool	Concentration A.U./10 ml CSF*
Heroin addicts	1	1.3
In withdrawal	2	0.6
Non-addicts	1	0.1
	2	0.1
	3	0.2

* 1 A.U. will increase the electrically induced twitch of the preparation by 30%.

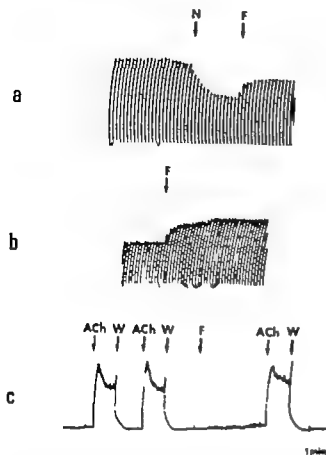


Fig. 1 Guinea-pig ileum longitudinal muscle preparation in Krebs-bicarbonate solution. In *a* and *b* it was stimulated electrically at a frequency of 0.1 Hz. The bath volume was 2.7 ml. (*a*) Recording of the reversal of the normorphine-induced suppression of the twitch. Normorphine (N) concentration was $\times 10^{-6}$ M. The antagonistic factor (F) originates from 100 ml control CSF. (*b*) Increase of the magnitude of the contraction by the factor (F) from 700 ml control CSF. (*c*) No effect of factor (F) from 700 ml of control CSF on the contraction produced by 3 μ g acetylcholine (ACh). (W)=wash.

absence of normorphine this fraction potentiated the twitch (Fig. 1*b*). The potentiation was not influenced by naloxone but was completely blocked by atropine.

The possibility of interaction between the antagonistic factor and exogenously applied acetylcholine was tested in a separate experiment. Acetylcholine at a dose of 3 μ g caused about the same contraction as that produced by the electric pulse. In the presence of the fraction there was no change in response (Fig. 1*c*). The same fraction showed weak affinity in a receptor assay with D-Ala¹ Leu⁵-enkephalin-H as radio-indicator: the amount of this fraction which had increased the twitch two-fold inhibited binding by 33%. The frac-

tion contained no material which was recognized by substance P-directed antibodies.

As illustrated in Table 1 particularly high concentrations of the antagonistic factor were found in CSF from narcotic addicts during abstinence.

The data presented here confirm the existence of a factor with apparent anti-opioid activity. However, since the factor described here also potentiates the electrically-induced contraction in a morphine-naïve ileum preparation it is not a pharmacologic antagonist. That is to say, it does not appear to compete with opioids for their receptors as naloxone does. By itself naloxone does not affect electrically induced contractions of guinea-pig ileum. Morphine inhibits contraction of the ileum myenteric plexus preparation which is thought to be a consequence of its ability to block release of acetylcholine (Paton 1957; Lees et al. 1977). The CSF factor may have the opposite effect, i.e. stimulating the release, although direct evidence is lacking at present.

The relation of the present antagonistic factor to the material isolated from brain and described by others to be "antiopioid" (Ungar & Ungar 1978; Han et al. 1979) is unclear since neither of these groups tested their material on a morphine-naïve preparation and because chemical identification is lacking. The elevated concentrations of the present factor in CSF from addicts indicates its potential functional significance.

This work was supported by the National Institute on Drug Abuse (USA) and the Swedish Medical Research Council. Dr Bengt Sjöstrand, University of Lund, Sweden kindly provided control CSF samples.

REFERENCES

- HAN C S, TANG J, HUANG B S. & ZHANG N H 1979 Acupuncture tolerance: Endogenous opiate substance implicated. *Chin Med J* 9: 63-67.
- LEES G M, KOSTERLITZ H W & WATERFIELD A A 1972 Characteristics of morphine-sensitive release of neuro-transmitter substances. In: *Agonist and antagonist actions of narcotic analgesic drugs* (ed. H W Kosterlitz, H O J Collier & J M Villarreal), pp. 142-157. The Macmillan Press Ltd.
- PATON W D M 1957 The action of morphine and related substances on contraction and on acetylcholine output of coxially stimulated guinea-pig ileum. *Br J Pharmacol Chemother* 12: 119-17.
- TERENIUS L 1974 A rapid assay for the narcotic receptor in rat brain: application to methadone analogs. *Acta Pharm Toxicol* 34: 88-91.

- TERENIUS, L. 1975 Opiate receptors and their ligands
in Proc. Soc. Int. Congr. Pharmacol. (ed. J. Tuomisto
and M. K. Paasonen) 1, 153-163.
- UNGAR, G. & UNGAR, A. L. 1976 Endogenous mor-
phine antagonist in morphine treated rat brain. Fed.
Proc. 35, 309.
- UNGAR, G., UNGAR, A. L., MALIN, D. H. &
SARANTAKIS, D. 1977 Brain peptides with opiate
antagonist action: their possible role in tolerance and
dependence. *Psychoneuroendocrinology* (1): 1-10.
- WAHLSTRÖM, A. & TERENIUS, L. 1980 Chemical
characteristics of CSF endorphins. *FEBS Lett.* 118,
241-244.

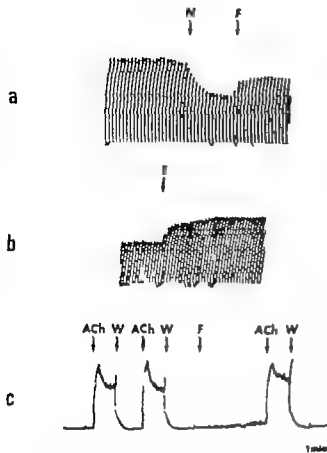


Fig. 1 Guinea-pig ileum longitudinal muscle preparation in Krebs-bicarbonate solution. In *a* and *b* it was stimulated electrically at a frequency of 0.1 Hz. The bath volume was 2.7 ml. (*a*) Recording of the reversal of the normorphine-induced suppression of the twitch. Normorphine (N) concentration was 2×10^{-6} M. The antagonistic factor (F) originates from 100 ml control CSF. (*b*) Increase of the magnitude of the contraction by the factor (F) from 100 ml control CSF. (*c*) No effect of factor (F) from 100 ml of control CSF on the contraction produced by 3 μ g acetylcholine (ACh). (W) = wash.

absence of normorphine this fraction potentiated the twitch (Fig. 1*b*). The potentiation was not influenced by naloxone but was completely blocked by atropine.

The possibility of interaction between the antagonistic factor and exogenously applied acetylcholine was tested in a separate experiment. Acetylcholine at a dose of 3 μ g caused about the same contraction as that produced by the electric pulse. In the presence of the fraction there was no change in response (Fig. 1*c*). The same fraction showed weak affinity in a receptor assay with D-Ala²-Leu⁵-enkephalin⁹ H as radio-indicator: the amount of this fraction which had increased the twitch two-fold inhibited binding by 33%. The frac-

tion contained no material which was recognized by substance P-directed antibodies.

As illustrated in Table 1 particularly high concentrations of the antagonistic factor were found in CSF from narcotic addicts during abstinence.

The data presented here confirm the existence of a factor with apparent anti-opioid activity. However, since the factor described here also potentiates the electrically induced contraction in a morphine-naïve ileum preparation, it is not a pharmacologic antagonist. That is to say, it does not appear to compete with opioids for their receptors as naloxone does. By itself, naloxone does not affect electrically induced contractions of guinea-pig ileum. Morphine inhibits contraction of the Bear myenteric plexus preparation, which is thought to be a consequence of its ability to block release of acetylcholine (Paton 1957; Lees et al. 1977). The CSF factor may have the opposite effect, i.e. stimulating the release, although direct evidence is lacking at present.

The relation of the present antagonistic factor to the material isolated from brain and described by others to be "antimorphine" (Ungar & Ungar 1976; Han et al. 1979) is unclear, since neither of these groups tested their material on a morphine-naïve preparation and because chemical identification is lacking. The elevated concentrations of the present factor in CSF from addicts indicates its potential functional significance.

This work was supported by the National Institute on Drug Abuse (USA) and the Swedish Medical Research Council. Dr Bengt Sjölund, University of Lund, Sweden, kindly provided control CSF samples.

REFERENCES

- HAN C S, TANG J, HUANG B S & ZHANG N H 1979. Acupuncture tolerance: Endogenous opiate substance implicated. *Chin Med J* 92, 623-627.
- LEES G M, KOSTERLITZ H W & WATERFIELD, A A 1972. Characterization of morphine-sensitive release of neuro-transmitter substances. In: *Agonist and antagonist actions of narcotic analgesic drugs* (ed. H W Kosterlitz, H O J Collier & J E Villarreal), pp. 142-152. The Macmillan Press Ltd.
- PATON W D M 1957. The action of morphine and related substances on contraction and on acetylcholine output of coxially stimulated guinea-pig ileum. *Br J Pharmacol Chemother* 12, 119-127.
- TERENIUS L 1974. A rapid assay for the narcotic receptor in rat brain: application to methadone analogs. *Acta Pharm Toxicol* 34, 83-91.

Characteristics of right ventricular receptors with non-medullated vagal afferents in the cat

PETER THORÉN

From the Department of Physiology, University of Göteborg, Sweden

There is a large population of left ventricular receptors responding to non-medullated vagal afferents in dogs (Coleridge et al. 1964; Muers & Sleight 1972) and cats. When activated, these receptors induce powerful depressor reflexes, characterized by bradycardia and vasodilatation (Thorén 1979).

These receptors are also the main receptor stations for eliciting the Bezold-Jarisch reflex (Thorén 1979).

The receptor discharge in relation to changes in cardiac dynamics was recently described by Thorén (1979) and the receptors seemed to respond mainly to changes in left ventricular end-diastolic pressure but not to isolated changes in ventricular systolic pressure. There also exist a sparse population of receptors with non-medullated afferents in the right ventricle of the dog (Coleridge et al. 1964; Muers & Sleight 1972). However, these authors did not describe the firing characteristics of the receptors.

The aim of the present study therefore was to examine the firing characteristics of right ventricular C-fibres with non-medullated vagal afferents. Cats, anesthetized with chloralose, were decannulated, the pericardium was opened and wires placed around the ascending aorta and the pulmonary artery. The right vagus was cut below the entrance of the cardiac nerve. This eliminated completely the afferent traffic from the pulmonary inflation receptors, which greatly facilitated the identification of and recording from, cardiac afferents.

Pressures were measured via catheters inserted into the aorta and right atrium and connected to a Beckman 230C transducers. The pressure in the right ventricle was measured via a thin needle inserted into the ventricle.

Thin filaments from the right cervical vagus were placed on an electrode for recordings of impulse activity as described previously by Thorén (1977). Conduction velocity was measured by application of an electrical stimulus to the right cardiac nerve and recording the evoked potential in the neck. As a screening procedure to find right ventricular C-fibres which normally have a low irregular discharge, the activity in all filaments dissected from the vagus was observed during a brief occlusion of the pulmonary artery. Localization of the receptors to the right ventricle was further established by the failure of the receptors to increase their firing during a balloon occlusion of the bicuspid valve and by probing of the heart with fine plastic probes.

The study is based on recordings from 8 receptors in 7 cats. The endings were located throughout the entire right ventricle. The conduction velocity in the cervical vagus was 0.8 ± 0.1 m/s (mean \pm S.E.) and the total conduction time from the receptor site to the recording electrode was from 115 to 250 ms. Two of the receptors had spontaneous activity during normal conditions, one ending in phase with the cardiac cycle and one ending with a low frequent irregular activity. The other endings were silent.

The receptor response to graded pulmonary artery occlusion was also examined. Upon activation 3 endings showed mainly irregular discharge with no obvious relation to the cardiac cycle but the other 5 receptors discharged with cardiac rhythmicity. When taking the total conduction time into account all these endings were activated in systole just as the endings in the left ventricle. The relation between the receptor activity and changes in right ventricular systolic and end-diastolic pressure is shown in Fig. 1. Upon a graded increase in right ventricular afterload there is a simultaneous increase in both systolic and end-diastolic pressure and a parallel increase in receptor discharge.

pressure, despite the fact that activation occurred during systole. The reason for this paradoxical finding is probably that changes in filling pressure of the left ventricle will markedly influence the following systole by the Starling mechanism as discussed by Thoren (1979). The reason why systolic changes in aortic pressure do not influence the receptor activity is probably that the left ventricle can stand small or moderate changes in afterload without changing the end-diastolic pressure due to the so-called barometric autoregulation.

Right ventricular receptors are also activated during systole indicating that the systolic contraction is of major importance for the receptor activation. However, the activity seems to increase parallel to the changes in both right ventricular systolic and end-diastolic pressure. The reason is probably that the right ventricle does not show any evident barometric autoregulation, meaning that even a fairly small or moderate change in the right ventricular systolic pressure will induce a clear rise in the right ventricular end-diastolic pressure.

One the conclusion from this study is that the characteristics of all the mechanosensitive C-fibre endings in the heart are very much dependent on the thickness of the myocardium in which the receptors are situated. Thus, left ventricular C-fibre endings do not change their activity upon moderate changes in left ventricular systolic pressure (Thoren 1979) but are fairly sensitive to changes in left ventricular end-diastolic pressure due to the fact that the left ventricle can stand small or moderate changes in systolic pressure without changing end-diastolic volume. In contrast C-fibres in the atria respond exactly to the atrial distension during the systole, simply because the receptor endings are

maximally deformed during the atrial filling. The right ventricular C-fibre endings are activated during the right ventricular systole because the receptor site is then maximally deformed. Due to the dynamics of the right ventricle the activity of the receptors correlates both with changes in right ventricular systolic and end-diastolic pressure.

This work was supported by the Swedish Medical Research Council project number 14X-04764 and 14X-00016.

REFERENCES

- AVIADO D. M., LILTH KALOW W., SCHMIDT C. F., TURNBULL G. L., PESKIN D. W. & HESS M. E. & WEISS A. J. 1951 Respiratory and circulatory reflexes from the perfused heart and the pulmonary circulation of dog. *Amer J Physiol* 165: 261-277.
- BARER, G. R. & KOTTAGODA S. R. 1958 Changes in heart rate and blood pressure of the cat in response to increased pressure in the right side of the heart. *J Physiol (Lond)* 143: 1-10.
- COLERIDGE, H. M., COLERIDGE, J. C. G. & KIDD C. 1964 Cardiac receptors in the dog, with particular reference to the types of afferent ending in the ventricular wall. *J Physiol (Lond)* 114: 323-339.
- MUERS, M. H. & SLEIGHT P. 1977 Action potentials from ventricular mechanoreceptors stimulated by occlusion of the coronary arteries in the dog. *J Physiol (Lond)* 221: 283-309.
- ROSS, J., FRAHM, C. J. & BRAUNWALD E. 1961 The influence of intracardiac baroreceptors on venous return, systemic vascular volume and peripheral resistance. *J Clin Invest* 40: 563-572.
- THOREN P. 1977 Characteristics of left ventricular receptors with non-medullated vagal afferents. *Circulat Res* 40: 415-421.
- THOREN P. 1979 Role of cardiac vagal C-fibres in cardiovascular control. *Rev Physiol Biochem Pharmacol* (Springer Verlag) 86: 1-94.

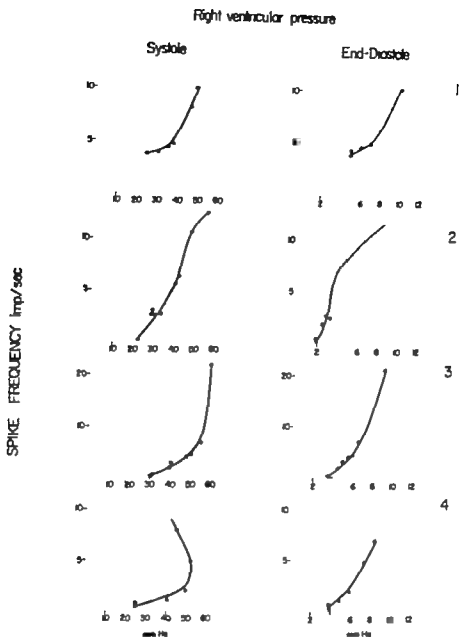


Fig 1 Pressure discharge curves for 4 right ventricular C-fibre endings during graded pulmonary artery occlusion. The activity for each receptor is plotted against the right ventricular systolic and end-diastolic pressure.

The present study describes C fibre endings determined on basis of conduction velocity measurements and originating in the right ventricle of the cat. However in relation to the large number of receptors earlier found in the left ventricle (Thorén 1977, 1979) the population of right ventricular C fibres seems to be small and this is also likely to be the reason why distension of the right ventricle induces only weak circulatory reflexes (Aviado et al 1951, Barer & Kottagoda 1958, Ross et al 1961). The relatively small muscle mass of the right in

comparison with the left ventricle might be the reason for the small numbers of endings.

When activated the right ventricular receptors as well as the left ventricular receptors display a cardiac rhythmicity in systole indicating that the systolic contraction of the ventricles is of major importance for the receptor activation. During aortic occlusion or transfusion the activity in the left ventricular C fibre endings correlated well with changes in left ventricular end-diastolic pressure but not with changes in the left ventricular systolic

pressure despite the fact that activation occurred during systole. The reason for this paradoxical finding is probably that changes in filling pressure of the left ventricle will markedly influence the following systole by the Starling mechanism as discussed by Thoren (1979). The reason why isolaute changes in systolic pressure do not influence the receptor activity is probably that the left ventricle can stand small or moderate changes in afterload without changing the end-diastolic pressure due to the so-called homeometric autoregulation.

Right ventricular receptors are also activated during systole indicating that the systolic contraction is of major importance for the receptor activation. However the activity seems to increase parallel with the changes in both right ventricular systolic and end-diastolic pressure. The reason is probably that the right ventricle does not show any evident homeometric autoregulation meaning that even a fairly small or moderate change in the right ventricular systolic pressure will induce a clear rise in the right ventricular end-diastolic pressure.

Thus the conclusion from this study is that the characteristics of all the mechanosensitive C-fibre endings in the heart are very much dependent on the dynamics of the myocardium in which the receptors are situated. Thus, left ventricular C-fibre endings do not change their activity upon moderate changes in left ventricular systolic pressure (Thoren 1979) but are fairly sensitive to changes in left ventricular end-diastolic pressure, due to the fact that the left ventricle can stand small or moderate changes in systolic pressure without changing end-diastolic volume. In contrast C-fibres in the atria respond mainly to the atrial distension during the rising, simply because the receptor endings are

maximally deformed during the atrial filling. The right ventricular C-fibre endings are activated during the right ventricular systole because the receptor site is then maximally deformed. Due to the dynamics of the right ventricle the activity of the receptors correlates both with changes in right ventricular systolic and end-diastolic pressure.

This work was supported by the Swedish Medical Research Council, project number 14X-04764 and 14X-00016.

REFERENCES

- AVIADO D M, LIT H, KALOW W, SCHMIDT C F, TURNBULL G L, PESKEN O W, HESS M E & WEISS A J 1951 Respiratory and circulatory reflexes from the perfused heart and the pulmonary circulation of dog. *Amer J Physiol* 165: 261-277.
- BARER, G. R. & KOTTAGODA, S. R. 1958. Changes in heart rate and blood pressure of the cat in response to increased pressure in the right side of the heart. *J Physiol (Lond)* 143: 1-10.
- COLERIDGE, H. M., COLERIDGE, J. C. G. & KIDD C. 1964. Cardiac receptors in the dog, with particular reference to two types of afferent ending in the ventricular wall. *J Physiol (Lond)* 174: 33-39.
- MUERS, M. F. & SLEIGHT P. 1972. Action potentials from ventricular mechanoreceptors stimulated by occlusion of the coronary sinus in the dog. *J Physiol (Lond)* 221: 283-309.
- ROSS J, FRAHM C J & BRAUNWALD E. 1961. The influence of intracardiac baroreceptors on venous return, systemic vascular volume and peripheral resistance. *J Clin Invest* 40: 463-472.
- THOREN P 1977. Characteristics of left ventricular receptors with non-medullated vagal afferents. *Circulat Res* 40: 415-421.
- THOREN P 1979. Role of cardiac vagal C-fibres in cardiovascular control. *Rev Physiol Biochem Pharmacol (Springer Verlag)* 86: 1-94.

Excessive cellular acidosis an important mechanism of neuronal damage in the brain?

STIG REHNCRONA, INGMAR ROSÉN and BO ■ SIESJÖ

Laboratory of Experimental Brain Research, and Department of Neurosurgery University Hospital S-221 ■ Lund, Sweden

There is evidence that in cerebral hypoxia/ischemia of sufficient severity (and duration) to induce neuronal cell damage, cellular energy metabolism is grossly perturbed. However, some results suggest that the damage incurred does not always correlate with the energy failure. For example, seemingly paradoxical results were obtained when metabolic recovery, as studied after 30 min of either complete, or severe incomplete ischemia (cerebral blood flow zero and <5% of control, respectively), in these experiments, in spite of a similar perturbation of cerebral energy state during ischemia, metabolic recovery was clearly better following complete ischemia (Nordström et al. 1978; see also Björnsdóttir & Kjellström 1973). It was tempting to conclude that, in incomplete ischemia, the scant supply of oxygen or substrate was responsible for the serious outcome. So far, attempts to prove the existence of oxidative breakdown of tissue constituents, e.g. through lipid peroxidation, have failed (Rehncrona et al. 1980, b), and recent interest has been focussed on the consequences of a continued glucose supply to the ischemic tissue.

In a previous study from this laboratory, an attempt was made to study the influence of acidosis on recovery following complete ischemia (Ljunggren et al. 1974). In this condition, the resulting lactic acidosis is determined by the preischemic stores of glucose and glycogen, which were varied by insulin injection (hypoglycemia) or glucose administration (hyperglycemia). In spite of the fact that, at the end of 5 min of ischemia, tissue lactic acid concentrations varied between about 5 and about 20 $\mu\text{mol/g}$, metabolic recovery was similar

that tissue lactic acid levels exceeding about 20 $\mu\text{mol/g}$ is detrimental for recovery (for literature see Myers 1979).

The present expts. were designed to study the influence of blood (and tissue) glucose levels on recovery following severe incomplete ischemia of 30 min duration. In addition, tissue glucose levels were similarly manipulated during complete ischemia.

Method The experiments were performed on male Wistar rats (250-350 g) that were either allowed free access to rat pellets and tap water or starved for 16-24 h prior to expts. After induction of anesthesia with halothane and operation under 0.6% halothane and 70% N_2O , the animals were maintained artificially ventilated on 70% N_2O and 30% O_2 , their body temperature being maintained at 37°C. Physiological parameters measured included blood pressure, pH and blood gases, as well as EEG and somatosensory evoked potentials.

Complete ischemia was induced by raising the CSF pressure above the systolic blood pressure by infusion of an artificial CSF solution into the cisterna magna, and incomplete ischemia by ligation of the carotid arteries combined with a lowering of mean arterial blood pressure to 50 mmHg (for details, see Nordström et al. 1978; Rehncrona et al. 1980, b). The period of ischemia was 30 min and recovery of 90 min duration was induced by discontinuation of CSF infusion and removal of carotid clamps with reinfusion of blood, respectively. Tissue was frozen for metabolic analyses at the end of ischemia and at the end of recovery (separate groups).

The following groups of animals exposed to complete ischemia were studied: () normally fed animals, (b) fasted animals infused i.v. with 2 ml of 50% glucose solution during 15 min prior to induction of ischemia and () fasted animals infused

In a recent series of expts. Myers and associates have reported that with more prolonged periods of hypoxia and ischemia, recovery varies with the nutritional state of the animals, and the results suggest

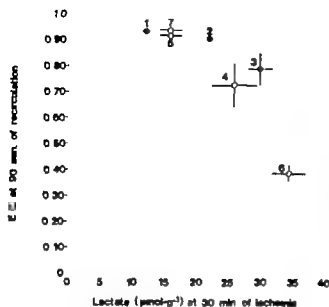


Fig. 1 The adenylate energy charge

$$E.C. = \frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]}$$

of rat cortical tissue at 90 min of recirculation following 30 min of cerebral ischemia as a function of the cortical lactate concentration at the end of ischemia. Experimental groups: ■ Non-ischemic controls ● reversible complete ischemia. 1 normally fed fasted infused with glucose prior to ischemia 3 fasted hypercapnic and infused with glucose prior to ischemia. 4 Reversible incomplete ischemia. 4 normally fed 5 fasted infused with Krebs-Henseleit solution prior to ischemia 6 fasted infused with glucose prior to ischemia 7 fasted infused with glucose during recirculation. There were 6 or 8 animals in each group. The symbols represent mean \pm S.E. Postischemic E.C. differed significantly ($P < 0.001$ Student's *t*-test) from controls in groups with ischemic lactate concentrations exceeding $20 \mu\text{mol/g}$.

similarly after $P_{a\text{CO}_2}$ had been increased to 100–120 mmHg. Hypercapnia was induced to further increase tissue glucose concentrations. The groups exposed to severe incomplete ischemia were as follows: (a) fed animals (b) fasted animals infused with 2 ml Krebs-Henseleit solution (c) fasted animals infused with 2 ml of a 50% glucose solution and (d) fasted animals given an equal amount of glucose at the end of the ischemic period. The latter group was included to exclude the possibility that an elevated glucose concentration during recirculation affected metabolic recovery.

Results and Discussion In all groups studied pre- and postischemic physiological parameters were similar and in all animals spontaneous or induced electrical activity ceased during ischemia. In all animals deterioration of cerebral energy state dur-

ing ischemia as evaluated from the concentration of PCr, ATP, ADP and AMP was similar. Pre-ischemic glucose concentrations varied between about $5 \mu\text{mol/g}$ (fasted animals) and $35 \mu\text{mol/g}$ (glucose-infused animals).

The main results are illustrated in Fig. 1 which relates the calculated energy charge of the adenine nucleotide pool at the end of the 90 min recirculation period to the tissue lactic acid concentration at the end of the 30 min period of ischemia. The following points emerge: (1) Following complete ischemia metabolic recovery is adversely affected if tissue lactic acid concentrations during ischemia are raised by glucose infusion at normal and increased CO_2 tension. (2) If animals are fasted recovery is at least as good following severe incomplete as following complete ischemia. In fact in the fasted animals extensive recovery of EEG and evoked potentials was observed. (3) Glucose infusion instituted before (but not after) the period of incomplete ischemia adversely affects recovery. (4) A detrimental effect of lactic acid is noted if its concentration exceeds $20\text{--}25 \mu\text{mol/g}$.

In summary our results suggest that if the tissue hypoxia is sufficiently severe to disrupt the phosphorylation state of the tissue, excessive lactic acidosis is detrimental to recovery. The results offer an explanation for the paradoxical results reported in the introduction and also seem to explain why other authors have failed to find that incomplete ischemia is more harmful than complete ischemia (Steen et al. 1979). Thus in these experiments fasted animals were used and the period of ischemia was limited to 10 min. However the mechanisms whereby acidosis disrupts cerebral metabolism remain to be defined.

This study was supported by grants from the Swedish Medical Research Council (14X 263) and from US PHS grant No. R01 NS07838 from N.I.H. Maj-Lis Smith is acknowledged for her skilful technical assistance.

REFERENCES

- HOSSMANN K. A. & KLEIHUES, P. 1973 Reversibility of ischemic brain damage. *Arch Neurol (Chic)* 29: 375–382.
- LJUNGGREN H., NORBERG K. & SIESJÖ B. K. 1974 Influence of tissue acidosis upon restoration of brain energy metabolism following total ischemia. *Brain Res* 77: 171–186.

- MYERS, R. E. 1979 A unitary theory of causation of anoxic and hypoxic brain pathology. In: *Cerebral hypoxia and its consequences* (ed. S. Fahn, J. N. Daro & L. H. Rowland). *Advances in neurology* Vol. 26, pp. 195-213. Raven Press, New York.
- NORDSTRÖM, C.-H., REHNCRONA, S. & SJESJÖ, B. K. 1974 Effects of phenobarbital in cerebral ischemia. Part two: Restoration of cerebral energy state as well as of glycolytic metabolites, citric acid cycle intermediates and associated amino acids after pronounced incomplete ischemia. *Stroke* 9: 335-343.
- REHNCRONA, S., FOLBERGROVA, J., SMITH, D. S. & SJESJÖ, B. K. 1980 Influence of complete and pronounced incomplete cerebral ischemia and subsequent recirculation on cortical concentrations of oxidized and reduced glutathione in the rat. *J. Neurochem.* 34: 477-486.
- REHNCRONA, S., WESTERBERG, E., ÅKESSON, B. & SJESJÖ, B. K. 1980a Brain cerebral fatty acids and phospholipids during and following complete and severe incomplete ischemia. *J. Neurochem.* (in press).
- STEEN, P. A., MICHENFELDER, J. D. & MILDE, J. H. 1979 Incomplete versus complete cerebral ischemia. Improved outcome with maximal blood flow. *Ann Neurol.* 6: 389-398.

Potential clamp of demyelinated rat nerve fibres

TOM BRISMAR

Department of Clinical Neurophysiology Karolinska sjukhuset, S-10401 Stockholm Sweden

Segmental demyelination was induced in the sciatic nerve of rats by intraneural injection of diphtheria toxin (Alt & Cavanagh 1969). Rats (300-400 g Sprague-Dawley) were anesthetized and given 0.5 U diphtheria toxin in 5 μ l buffered sterile isotonic NaCl solution by microinjection into the exposed sciatic nerve. About 14 days later the rats were sacrificed and the nerve was investigated. Pronounced segmental demyelination was seen in the fibres at the site of injection. A large diameter (>10 μ m) fibre with only paranodal demyelination was selected and dissected free from the rest over the neighbouring microelectrodes. The fibre was photographed and then mounted in the recording chamber for the potential clamp analysis (Brismar 1980).

Fig 1 shows fibre with paranodal demyelination obtained in this way. The membrane currents of this fibre are shown in Fig 2. Positive potential steps were associated with comparatively small inward currents and the reversal potential for the

initial current was low (about 0 mV). The delayed outward currents and the inward current tails were considerably larger than in normal fibres.

The ionic specificity of the membrane currents was tested by exchange of the external Ringer solution to a solution with high [K]. In this solution the initial inward current was negligible and the delayed currents became inward at potentials below -10 mV and outward at more positive potentials. The delayed currents were blocked in 5 mM TEA (tetra ethyl ammonium chloride).

The membrane leak conductance (measured from the current associated with negative potential steps and calculated for an assumed nodal width of 1.0 μ m, i.e. for an unchanged nodal surface area) was 150 mS cm⁻² that is similar to that in normal fibres. The membrane time constant (measured from the recorded membrane potential change associated



Fig 1 Paranodal demyelination in rat nerve fibre. Photomicrograph (LEITZ inverted microscope, dark field illumination) of fibre from sciatic nerve 14 days after diphtheria toxin injection. Arrows show demyelinated segments.

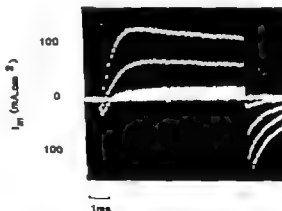


Fig 2 Membrane currents (I_m) of potential clamped demyelinated rat nerve fibres (shown in Fig 1). Holding potential -80 mV, positive potential pulses to +60, -40 and -20 mV respectively associated traces of membrane current superimposed in the figure. External Ringer solution: 147 mM NaCl, 5.9 mM KCl, 3.1 mM CaCl₂, 5.0 mM Tris-SIGMA pH 7.4. Current calibration tentatively performed as for normal fibres, for a nodal gap width 1.0 μ m (Brismar 1980). Temp. 24°C.

with a current pulse) was 0.55 ms which was almost 10 times longer than normally suggesting an increase in the nodal capacitance.

It is concluded that paranodal demyelination was associated with an increase in the axoplasmic $[Na]$ and an increase in the delayed K-permeability which normally is small in rat fibres (Brismar 1980). A similar increase in the delayed currents was described in fibres of rats with alloxan induced diabetes (Brismar 1979). There is further evidence of an increase in nodal capacitance related to the demyelination whereas the nodal resistance at resting potential was little affected.

Summary Potential clamp analysis of paranodally demyelinated nerve fibres (induced by diphtheria toxin) showed decreased inward Na-currents con-

siderably increased delayed K-permeability and increased nodal membrane capacitance.

This work was supported by the Foundations of the Karolinska Institute, the Swedish Diabetic Association and the Swedish Medical Research Council (Project N 14X-4255).

REFERENCES

- ALLT G & CAVANAGH, J H 1969 Ultrastructural changes in the region of the node of Ranvier in the rat caused by diphtheria toxin. *Brain* 92: 459-468.
- BRISMAR T 1979 Potential clamp experiments on myelinated nerve fibres from alloxan diabetic rats. *Acta Physiol Scand* 105: 384-386.
- BRISMAR T 1980 Potential clamp analysis of membrane currents in rat myelinated nerve fibres. *J Physiol (Lond)* 298: 171-184.

Autoinhibition versus transsynaptic control of noradrenaline secretion in the rabbit kidney

PER HEDQVIST

Department of Physiology, Karolinska Institutet, S-10401 Stockholm, Sweden

Ample experimental evidence indicates the presence of presynaptic α -adrenoceptors, through which the neurotransmitter noradrenaline (NA) is believed to restrict its own release (cf. Langer 1979). In addition to these NA autoreceptors, there seems to be other presynaptic receptors which mediate feedback control of NA release by prostaglandins and purines, substances preferentially derived from postsynaptic elements (cf. Hedqvist 1977; Fredholm & Hedqvist 1980). In this communication data are presented, which indicate that transsynaptic feedback substantially contributes to the control of NA secretion, being at least as effective as NA autoregulation, in the rabbit kidney.

Methods. Rabbits of either sex, weighing 1.5–2.5 kg were anesthetized with pentobarbital and the left kidney with its nerves and vascular supply was isolated and perfused at a constant rate of 10 ml/min with a slightly modified Tyrode's solution, as previously described (Hedqvist, Fredholm & Ölundh 1978). The experiment was started with a 15 min intrarterial infusion of ^3H -(-)-NA (25 μCi , 40 nCi/nmol; Amersham) or ^3H -adrenaline (5 μCi , 296 nCi/nmol; Amersham) followed by a 30 min washout period. Thereafter the renal nerves were stimulated by means of platinum electrodes and a Grass S4 stimulator delivering trains of pulses (3 Hz, 1 ms, 10 V) at intervals of usually 10 min. The perfusate was collected in 1 min fractions and the radioactivity was determined by counting 1 ml samples in 5 ml of Picofluor in a tri-carb liquid scintillation spectrometer. The values were expressed as counts/min. Quenching was monitored by external standardization.

Results and Discussion. After labelling of the kidney with ^3H -(-)-NA, phentolamine (6 μM) increased the outflow of tracer in response to renal nerve stimulation (3 Hz, 60 s) by $87 \pm 8\%$ (Mean \pm S.E., $n=10$), and blocked the ensuing vaso-

constrictor response. Raising the phentolamine concentration to 15 μM did not cause any additional increase in outflow of tracer. It has been shown that more than 80% of the radioactivity overflowing in response to nerve stimulation is accounted for by intact H-NA in the kidney (Frame & Hedqvist 1975). It is currently believed that phentolamine increases transmitter overflow by blocking presynaptic α -adrenoceptors that mediate NA autoinhibition while its inhibitory effect on NA uptake seems to be of little importance in this respect (cf. Langer 1979).

It has been shown that α -adrenoceptor antagonists substantially reduce or even abolish the release of prostaglandin E_2 and adenosine in the rabbit kidney (Fredholm & Hedqvist 1978; Hedqvist et al. 1980). Both these substances are potent inhibitors of NA release in the rabbit kidney (Frame & Hedqvist 1975; Hedqvist & Fredholm 1976). It is not unlikely therefore that α -adrenoceptor manipulation tends to overestimate the significance of NA autoinhibition because of simultaneous interference with a feedback mechanism, operated through prostaglandin E_2 and adenosine.

In order to evaluate the relative significance of the different control mechanisms, the stimulant effect of phentolamine on NA secretion was compared with that of the prostaglandin synthesis inhibitor indomethacin, and the adenosine antagonist theophylline. Indomethacin (6 μM) and theophylline (100 μM) increased nerve-induced release of NA by 27 and 34% respectively (Fig. 1) in harmony with previous observations in this tissue (Frame & Hedqvist 1975; Hedqvist et al. 1978). It is true that theophylline is a phosphodiesterase inhibitor but it is highly unlikely that such an effect could contribute significantly to its enhancing effect on NA release. Thus, 100 μM theophylline inhibits rabbit kidney phosphodiesterase by less than 25%

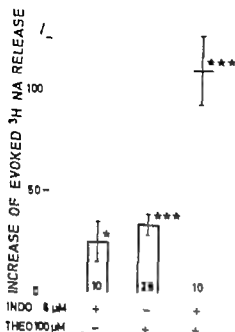


Fig. 1 Effects of indomethacin (INDO) and theophylline (THEO) alone and in combination, on ³H(-)-NA release evoked by nerve stimulation (3 Hz 60 s) in Tyrode's perfused rabbit kidneys. Mean values ± S.E., number of experiments indicated in the columns. $P < 0.05$, $P < 0.001$.

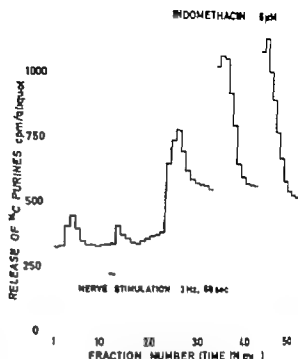


Fig. 2 Tyrode's perfused rabbit kidney, prelabelled with ¹⁴C-adenine. Effect of indomethacin on basal and nerve induced (3 Hz 60 s) release of tracer, predominantly in the form of adenosine, inosine and hypoxanthine.

and other more effective phosphodiesterase inhibitors do not increase NA release in the kidney (Hedqvist et al 1978). When indomethacin and theophylline were given together the nerve induced release of NA increased significantly more ($P > 0.05$) than could be accounted for by the sum of their individual effects (Fig. 1). A possible explanation for this synergistic effect was given in experiments in which purine release was studied.

It has been shown (Fredholm & Hedqvist 1978) that infusion of labelled adenine into the renal artery results in a substantial uptake and incorporation of the label, predominantly in the form of nucleotides, while subsequent release, both spontaneously and that induced by nerve stimulation, occurs as nucleosides, adenosine and its metabolites, inosine and hypoxanthine. After labelling of the kidney with ¹⁴C-adenine, indomethacin caused a marked increase in the release of tracer, in all probability consisting of adenosine and its metabolites (Fig. 2). Both spontaneous release and that induced by renal nerve stimulation were augmented, and the effect was not specific for indomethacin, but readily occurred also with two other prostaglandin synthesis inhibitors, meclofenamic acid and ecicosatet.

These findings suggest that removal of a prostaglandin mediated mechanism, operated to restrict NA release and to attenuate vasoconstrictor responses, results in increased vascular resistance, in turn leading to adenosine release and inhibition of NA secretion. As a consequence, the use of prostaglandin synthesis inhibitors seems to represent an underestimate of removal of a prostaglandin mediated feedback mechanism operated on NA secretion in the kidney. Presently, it is not known whether the same holds true also for other tissues. However, prostaglandin synthesis inhibition is commonly associated with an increase in smooth muscle tone, which is a significant stimulus for purine release (cf. Hedqvist 1977; Fredholm & Hedqvist 1980).

Summing up, the results indicate that NA secretion may be controlled by the transmitter itself as well as by prostaglandins and purines. Since prostaglandins and purines are predominantly released from postfunctional elements, they represent a transsynaptic mechanism for feedback control of NA secretion, which seems particularly suitable to meet local tissue demands. Moreover, transsynaptic modulation seems to be at least as important as:

NA autoregulation, even when disregarding that manipulation of α -adrenoceptors presumably affects both mechanisms.

Supported by grants from the Swedish Medical Research Council (project nr 00-4342), Magnus Bergvall Foundation, and Karolinska Institutet.

REFERENCES

- FRANK, M. H. & HEDQVIST P. 1975 Evidence for phosphate mediated prejunctional control of renal vascular sympathetic tone. *Br J Pharmacol* 54 189-194.
- FREDHOLM, B. B. & HEDQVIST P. 1978 Release of 3H -amines from 3H -adrenine labelled rabbit kidney following sympathetic nerve stimulation, and its inhibition by α -adrenoceptor blockade. *Br J Pharmacol* 64 239-245.
- FREDHOLM, B. B. & HEDQVIST P. 1980 Modulation of neurotransmission by purine nucleotides and nucleosides. *Biochem Pharmacol* 29 1635-1643.
- HEDQVIST P. 1977 Basic mechanisms of prostaglandin action on autonomic neurotransmission. *Ann Rev Pharmacol Toxicol* 17 259-279.
- HEDQVIST P. & FREDHOLM B. B. 1976. Effects of adenosine on adrenergic neurotransmission. Prejunctional inhibition and postjunctional enhancement. *Naunyn-Schmiedeberg Arch Pharmacol* 293 217-223.
- HEDQVIST P., FREDHOLM B. B. & ÖLUNDH B. 1978 Antagonistic effects of theophylline and adenosine on adrenergic neuroeffector transmission in the rabbit kidney. *Circ Res* 43 597-598.
- HEDQVIST P., GUSTAFSSON L., HJEMDAHL, P. & SVANBORG, K. 1980. Aspect of prostaglandin action on autonomic neuroeffector transmission. In: *Advances in prostaglandin and thromboxane research* (ed B. Samuelsson, P. W. Ramwell & R. Paoletti), vol 8, pp 1-45-1248. Raven Press, New York.
- LANGER, S. Z. 1979 Physiological and pharmacological role of presynaptic receptor systems in neurotransmission. *Adv Biol* 18.13-22.

Specific sensations evoked by activity in single identified sensory units in man

H. ERIK TOREBJÖRK and JOSÉ L. OCHOA

Department of Clinical Neurophysiology, University Hospital, Uppsala, Sweden and
Department of Neurology, Dartmouth Medical School, Hanover, USA

The use of Jacques insulated tungsten semimicro-electrodes for single nerve fiber recordings in awake human subjects (Vallbo & Hagbarth 1968) largely materialized the desire to compare both stimulus and sensation with the messages which pass up the sensory nerve fibres (Adrian 1931). Out of the range of scientific accomplishments immediately anticipated from the application of the microelectrographic technique a few are still awaited, like for example a definitive answer to the questions—does activity in single sensory units of various types reach consciousness? and are the various sensory modalities encoded in single units? Uncertainty in this regard derives from the fact that various natural stimuli that can be applied to the skin normally activate several units with adjacent or overlapping receptive fields (Johansson & Vallbo 1974), thus confusing the experimental situation. Through the application of a technique to induce trains of impulses in single nerve fibers we have found it possible to compare sensation with the messages which pass up single sensory units in awake human subjects and thus demonstrate specific conscious percepts related to impulse activity in identified single sensory units in man.

The technique is based upon *intraneural microstimulation* and subsequent recording, through the same electrode, of the receptor characteristics and conduction velocity of individual sensory units. Our observations indicate that it is possible to activate single nerve fibers by this technique as it is possible to record impulses from single fibers. Confidence about fulfilment of the single unit requisite is gained from the observation of stringent criteria. Having ensured that, after nerve impalement, the electrode tip allows very selective and stable A and C single nerve fiber recording, the examiner explores minutely the nerve while delivering initially a 1 Hz train of short (0.25 ms) pulses at a learned suprathreshold intensity (0.3–0.9 V). The subject, instructed to report the occurrence of sensory events

referred to the territory of the pertinent nerve eventually signals some faint sensation. When such sensation is focally localized in a single and discrete skin area, the examiner upon delivery of natural stimuli to the skin is consistently able to identify in that area and usually nowhere else, the receptive field of a sensory unit whose evoked afferent impulses can be picked up from the tip of the exploring electrode now used for recording. Although natural stimulation of the receptive field must co-activate several units, usually only the protagonist unit which is in focus for the electrode can be recorded (Fig. 1). Intensity of the repetitive intraneural stimulus is then minimized into a region just above perceptual threshold where a pure sensation is captured, like pressure vibration, etc. with a pure temporal dimension which can be either continuous or intermittent. An all or nothing behaviour for the percept is demonstrated when the pure focal sensation does not grow in intensity nor area within a small range of stimulus intensities, thus reflecting unitary excitation. Proof that the fiber stimulated and the fiber recorded are one and the same can be obtained by physiologically marking the stimulated fiber prolonged, high frequency intraneural microstimulation can render that single myelinated sensory fiber hyperexcitable (Torebjörk & Ochoa, unpublished) as it does with motor fibers (Bergmans 1973). Upon intraneural recording this fiber will either discharge spontaneously or will generate a burst in response to a triggering pulse. Impulses evoked from the receptive field of that particular unit only will now interfere with or trigger activity in the hyperexcitable unit (Fig. 1). Thus, it can be certified that a recorded unit with defined receptor characteristics and conduction velocity is identical with the unit stimulated in the nerve. By this manoeuvre the identity has been proven for 51 mechanoreceptive units, classified as rapidly adapting (RA) Pacinian corpuscle units (PC) or slowly adapting units of various types (SA I, SA II).

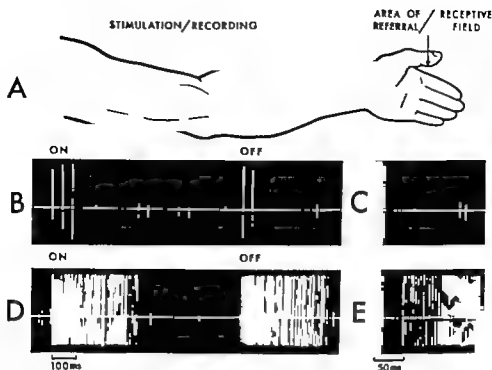


Fig. 1 Microelectrode stimulation and recording from the same single sensory unit (A) Intraneural microstimulation in the median nerve at elbow is referred as flutter vibration to a focal area in the thumb (B) Intraneural recording detects on and off responses in a single RA unit to skin indentation in the area of referral (C) Electrical stimulation in the receptive field of the RA unit excites a fiber with a conduction velocity of 45 m/s (White arrow indicates stimulus artifact.) (D-E) Prolonged intraneural microstimulation at high frequency induces post-tetanic hyperexcitability which marks the stimulated fiber. Mechanical (D) and electrical (E) stimuli in the receptive field of the RA unit now evoke stereotyped bursts of impulses. Time bases are common for B-D and for C-E respectively.

It will be realized that the intraneural microstimulation method for functional isolation of single afferent units selects for those that do arouse a conscious percept and if as long suspected there are units whose individual activity never reaches consciousness or units who only do so within a special frequency range they would pass undetected. For this reason the method is complemented by the routine identification of single units by conventional means where rather than primarily microstimulating units intraneurally and selecting by percept the examiner primarily excites units by natural stimulation of receptors while recording intraneurally thus selecting by electrophysiological criteria. Having established the response characteristics of an isolated unit the electrode is used to stimulate its fiber and the percept is established while depriving the subject of audiovisual cues as to the stimulus parameters.

From the combination of methods it has emerged that trains of impulses in single RA units in the

glabrous skin of the hand usually reach consciousness whereas activity in single SA II units practically never does. Fairly often trains of impulses in single SA I units can be perceived. The situation for C nociceptor units is less certain because of the natural conglomeration of their axons in bundles within Schwann cells and their frequent association with A delta fibers within nerve fascicles (Ochoa 1976). Thus although excitation of C fibers and A delta fibers has usually aroused conscious sensation it is difficult to ascertain whether the percept induced by one or several units.

Together with localizing an exclusive receptive field the subject of course volunteers a perceptual quality. Its description is concrete to begin with (painless-painful, intermittent-continuous, dull-bright) and then qualified (pressure vibration, stinging pain, dull pain, itch). The trained subject soon learns to predict accurately the response characteristics of the protagonist sensory units. For example, painless tapping which becomes inte-

almost better or vibratory buzzing at higher stimulation frequencies, is typically underlined by activity in RA (or PC) units with fast conducting myelinated fibers, as claimed by Talbot et al. (1968) in collateral experiments with monkey and man. Similarly painless continuous pressure is evoked by repetitive impulses in SA-I units with fast fibers. Sharp stinging pain has so far been found served by high threshold nociceptive units with slow myelinated fibers in the A delta range, and dull pain by low-threshold nociceptive units with conduction properties typical of unmyelinated (C) afferent fibers (Torebjörk 1974). There is nothing bizarre to the quality of the sensations evoked intraneurally: they are comparable to the familiar ones elicited via receptors by adequate stimuli. But the qualities of the sensory modalities experienced after natural stimulation, and the qualities of the sensations evoked when receptors are bypassed by intraneural microstimulation are not equivalent. For instance the microstimulated tactile sensation evoked from RA units is devoid of any pressure component, and the constant pressure sensation evoked by 10 Hz stimulation of SA-I units is devoid of tactile intermittency. This means that the percepts induced by intraneural microstimulation of single sensory units are pure and specific, whereas sensory modalities evoked by natural stimuli to the skin, like touch, may be a blend from activity in sensory units of more than one type.

A preliminary note on the sensations aroused from microstimulation in motor (muscle) nerve fascicles is pertinent. Tension and dull pain can be produced deeply from muscle but the physiological demonstration of the corresponding afferent units remains to be established. Occasionally pain is referred not only to the appropriate muscle territory. For instance, microstimulation in a median nerve fascicle innervating the flexor muscles in the forearm caused pain perceived as arising also from the carpal region. Such remote referral obviously implies convergence along the pain pathways, has not been observed when stimulating cutaneous fascicles in the upper limb.

As predicted by Adrian & Zotterman (1926), modulation of impulse frequency in a single unit may be enough to signal intensity of a stimulus. Indeed, firing frequency is translated into intensity of conscious percepts (pressure, pain) but determines the frequency of intermittent percepts (flutter-vibration). The sensory modality does not change with

frequency. The irrelevance of frequency modulation. The sensory modality does not change with frequency. The irrelevance of frequency modulation in terms of sensory modality coupled with the specific sensations evoked by impulse activity in single units of different types, as defined by their receptor characteristics, provides definitive proof for specificity in the sensory system while further questioning the Pattern Theory (Slovicar 1955).

It is anticipated that the technique of intraneural microstimulation will facilitate the identification of various types of sensory units in micro-neurographic recordings in man, and that it will provide a powerful tool to study the peripheral determinants for spatial, temporal and intensity resolution of sensations from skin and muscle.

This work was supported by the Swedish Medical Research Council (B 81:14X-05206-04B, K 80-14V 5822-01) and the Muscular Dystrophy Association of America.

REFERENCES

- ADRIAN E. D. 1931. The messages in sensory nerve fibres after their interpretation. *Proc Roy Soc B* 109: 1-18.
- ADRIAN E. D. & ZOTTERMAN Y. 1926. The impulses produced by sensory nerve endings. Part III. Impulses set up by touch and pressure. *J Physiol (Lond)* 61: 463-483.
- BERGMANS J. 1973. Physiological observations on single human nerve fibres. In: *New developments in electrophysiology and clinical neurophysiology* (ed. J. E. Desmedt) vol. 2, pp. 89-127. Karger, Basel.
- JOHANSSON R. & VALLBO Å. B. 1976. Skin mechanoreceptors in the human hand: an inference of some population properties. In: *Sensory functions of the skin in primates with special reference to man* (ed. Y. Zotterman) pp. 171-184. Pergamon Press, Oxford.
- OCHOA, J. 1976. The unmyelinated nerve fibre. In: *The peripheral nerve* (ed. M. N. London) pp. 106-158. Chapman and Hall, London.
- SINCLAIR, D. C. 1955. Cutaneous sensation and the doctrine of specific energy. *Brain* 78: 584-614.
- TALBOT W. H., DARIAN-SMITH I., KORNHUBER, H. H. & MOUNTCASTLE, V. B. 1968. The sense of flutter-vibration: comparison of the human capacity with response patterns of mechanoreceptive afferents from the monkey hand. *J Neurophysiol* 31: 301-334.
- TUREBJÖRK H. E. 1974. Afferent C units responding to mechanical, thermal and chemical stimuli in human non-glabrous skin. *Acta Physiol Scand* 92: 374-390.
- VALLBO Å. B. & HAUGBARTH K. E. 1968. Activity from skin mechanoreceptors recorded percutaneously in awake human subjects. *Exp Neurol* 21: 270-289.

Leukotriene B₄: A highly potent and stereospecific factor stimulating migration of polymorphonuclear leukocytes

CURT L. MALMSTEN, JAN PALMBLAD, ANN MARIE UDÉN, OLOF RÄDMARK, LAIS ENGSTEDT¹ and BENGT SAMUELSSON

¹Department of Chemistry, Karolinska Institutet, S-10401 Stockholm, Sweden and ²Department of Medicine IV, Karolinska Institutet, Södersjukhuset, S-10064 Stockholm, Sweden

Arachidonic acid is transformed via the cyclo-oxygenase catalyzed pathway into prostaglandins (including prostacyclin) and thromboxanes (Samuelsson et al. 1978). Recently a novel group of arachidonic acid derived compounds, the leukotrienes (LT), has been discovered (Samuelsson et al. 1980, Samuelsson & Hammarström 1980). Thus 5-hydroperoxy-eicosatetraenoic acid (5-HPETE) formed by lipoxygenase catalyzed oxygenation of arachidonic acid at C 5 can be transformed into the unstable epoxide intermediate leukotriene A₄ (LTA₄). Enzymatic hydrolysis of LTA₄ yields LTB₄ whereas two 5,11-dihydroxy isomers (compounds I and II) are formed by non-enzymatic hydrolysis of the intermediate LTC₄ which has SRS-A activity and is formed from LTA₄ by addition of glutathione. LTC₄ can be further metabolized by elimination of the γ-glutamyl residue into LTD₄. LTC₄ and LTD₄ have been identified in SRS-A from various sources. They are both potent bronchoconstrictors and increase the permeability of microvasculature (Giolini et al. 1980).

We have earlier demonstrated formation of 5-HETE, LTB₄ (including the two non-enzymatically formed isomers) and LTC₄ in human leukocytes (Samuelsson et al. 1980, Hansson & Rådmark 1980). In view of the earlier reported effects of lipoxygenase products on chemotaxis in human leukocytes (Goettl 1980) we have studied the effects of the various arachidonic acid derived products on chemotaxis and the response of human PMNLs to the chemotactic peptide fMLP in the presence of cyclo-oxygenase and lipoxygenase inhibition.

Leukocyte preparation. The erythrocytes in heparinized blood (10 IU heparin, without preservative, per ml blood) were sedimented in 4.5%

dextran T 500 and the supernatant withdrawn and centrifuged at 500×g for 6 min. The cellular pellet was washed twice in heparinized 0.9% saline (1 IU heparin per ml saline) and the leukocytes were suspended in a 3.1% HEPES buffer (Sigma, St. Louis, Mo., USA) dissolved in Hank's medium to make appropriate PMNL concentrations. In some experiments PMNLs were incubated with indomethacin (Sigma) or eicosatetraenoic acid (a kind gift from Dr J. Pike, Upjohn Co., Kalamazoo Mich., USA) for 15 min at 37°C both dissolved in ethanol at a final concentration of 0.5%. As controls served cells incubated with solvent only.

Preparation of 5-hydroxy-eicosatetraenoic acid (5-HETE), 5,12-dihydroxy eicosatetraenoic acids (LTB₄ and compound I and II) and LTC₄. Human PMNLs were incubated with arachidonic acid (Nu-Chek Prep. Inc. Elysian Minn. USA) and ionophore A23187 (Eli Lilly Indianapolis Ind. USA). The incubate was extracted and purified by high pressure chromatography as previously described (Borgeat & Samuelsson 1979, Rådmark et al. 1980).

Random migration and stimulated locomotion were assayed with a modification of a method measuring PMNL migration under agarose (Afzelius et al. 1980). Six series, each consisting of 3 wells, were punched in the agarose (containing 0.08% albumin). The diameter of one well was 3 mm and the distance between the centers of two adjacent wells was 5 mm. Ten μl of PMNL suspension

Abbreviations: fMLP=fornyl-Met-Leu-Phe, PMNL()=polymorphonuclear leukocytes, ETYA=5,8,11,14-eicosatetraenoic acid, SRS-A=slow reacting substance of anaphylaxis, 5-HETE=5-hydroxy-eicosatetraenoic acid, LT=Leukotriene

Table 1 The ability of LTB₄, LTC₄, 5-HETE and compounds I and II to induce a stimulated migration of neutrophils measured as the distance to the leading front cells in mm

The unstimulated migration never exceeded 0.13 mm in any examination. Mean \pm S.D. values for PMNLs from 5 different subjects (each sample triplets)

Conc. (M)	LTB	LTC ₄	5-HETE	Compound I	Compound II	fMLP
10 ⁻⁴	0.61 \pm 0.09*	0.17 \pm 0.07	0.18 \pm 0.03	0.66 \pm 0.30*	0.74 \pm 0.18*	-
10 ⁻⁵	0.81 \pm 0.05	0.14 \pm 0.04	0.13 \pm 0.01	0.43 \pm 0.07*	0.71 \pm 0.14*	-
10 ⁻⁶	0.56 \pm 0.07*	0.13 \pm 0.02	0.13 \pm 0.05	0.10 \pm 0.01	0.20 \pm 0.02*	0.80 \pm 0.27**
10 ⁻⁷	0.27 \pm 0.01	0.1 \pm 0.02	0.10 \pm 0.03	0.13 \pm 0.04	0.13 \pm 0.04	-
10 ⁻⁸	0.13 \pm 0.07	0.11 \pm 0.04	0.14 \pm 0.04	0.11 \pm 0.03	0.11 \pm 0.03	-

* $P < 0.01$ * $P < 0.05$ in comparison with measures for unstimulated cells (Wilcoxon's signed rank test).

PMNLs showed a desensitized pattern of stimulated migration (Chenoweth et al. 1979)

* Mean of triplets

containing approximately 10×10^6 cells/l was filled in the central well. The chemotactic factor either fMLP (10^{-7} M) in Hank's medium, 5-HETE or the leukotrienes dissolved in 10% ethanol were filled in the outer well. Tissue culture medium was added to the inner well.

The dishes were incubated for 0.5–3 h at 37°C in an atmosphere of 5% CO₂. After fixation with methanol and staining with hematoxylin and fuchsin the agarose dishes were removed and the distance migrated by the front was measured by microscopy and given in mm.

The orientation of lamellipodia and nuclei of 300 PMNLs migrating towards the cytotoxin and tissue medium wells was estimated by microscopy. The cells were considered polarized in the gradient when the nuclei were in the rear and the anterior lamellipodia located within a 90° sector open towards the cytotoxin well (Afzelius et al. 1980).

Effects of incubation of PMNLs with indomethacin or eicosatetraynoic acid (ETYA) When migration of PMNLs was stimulated with 10^{-7} M fMLP preincubation for 15 min with indomethacin (2.5×10^{-4} – 5×10^{-6} M) caused dose dependent inhibition of migration. Spontaneous migration was also slightly impaired ($P < 0.05$). However when the concentration of indomethacin was decreased to 2.5×10^{-6} M a significant enhancement of fMLP stimulated migration was observed ($P < 0.01$ Wilcoxon's signed rank test). In concentrations 2.5×10^{-7} M or below indomethacin showed no significant effect on stimulated or spontaneous migration when compared to controls.

PMNLs pretreated with ETYA for 15 min at 37°C showed markedly impaired migration when final inhibitor concentration was 2.5×10^{-4} – 2.5×10^{-6} M.

However at 2.5×10^{-4} M ETYA many PMNLs seemed damaged according to results of trypan blue exclusion. At final concentrations 2.5×10^{-6} M or below the results were comparable to those of control cells. This occurred both when migration was spontaneous or stimulated with 10^{-7} M fMLP.

Effects of 5-HETE, LTC₄, LTB₄ and compounds I and II When the different compounds to be tested were added to the cytotoxin well PMNL migration was significantly stimulated by LTB₄ ($P < 0.01$ Wilcoxon's signed rank test) and compounds I and II ($P < 0.05$) (Table 1). When 5-HETE or LTC₄ were utilized the migration was not significantly different from that of control cells.

In order to assess whether LTB₄ and compounds I and II stimulated directed or random migration i.e. chemotaxis or chemokinesis we analyzed the course of migration and the degree of orientation at 30, 60 and 120 min after the start of incubation and compared this with fMLP as cytotoxin. PMNLs stimulated by fMLP showed both the highest degree of orientation and progression of the cell front at 30 and 60 min. The orientation decreased after 120 min to control values (reference range (± 2 S.D.) is 13.5–30.9% Palmblad et al. to be published).

PMNLs stimulated by LTB₄ also showed directed and stimulated migration at 30 and 60 min when concentrations were 10^{-4} – 10^{-6} M. At 10^{-4} M LTB₄ decreased the stimulated migration presumably by causing desensitization (Chenoweth et al. 1979).

With compound I directed migration was observed at concentrations of 10^{-4} – 10^{-6} M and with compound II at concentrations of 10^{-4} – 10^{-7} M in the system employed.

The observation that eicosatetraynoic acid

(ETYA) caused dose-dependent decrease of PMNL migration induced by FMLP indicates that oxygenation of arachidonic acid is essential for the chemotactic response of human PMNLs to the peptide. High concentrations of indomethacin also inhibited the chemotactic effect of FMLP where as lower concentrations of the cyclo-oxygenase inhibitor increased the chemotactic response. These findings were compatible with a role of lipoxygenase products in chemotaxis and when some recently discovered metabolites of arachidonic acid in PMNLs (5-HETE, LTC, LTB and compounds I and II) were studied LTB was found to be a potent chemotactic factor. These findings confirm and extend recent reports from other laboratories demonstrating chemotactic effects of LTB (Ford-Hutchinson et al. 1980; Goetzl 1980). The non-enzymatic isomers of LTB (compounds I and II) were considerably less active than the enzymatically formed isomer of LTB and 5-HETE and LTC, did not show any significant effect on the migration in the system employed. The stereospecificity in the response to the dihydroxy acids provides support for physiological role of LTB as a chemotactic agent. The present results and recent studies thus indicate that the leukotrienes play a role not only in immediate hypersensitivity reactions but also in inflammation through the increase in permeability of microvasculature by LTC and LTD and the chemotactic effect of LTB.

This study is supported by grants from the Swedish Defence Research Institute and The Swedish Medical Research Council (project 03X-17 and 03X-03991). The skilful technical assistance of Mrs I Friberg and N Venizelos, M Sc, is gratefully acknowledged.

REFERENCES

- AZELIUS, B. A., EWERTS L., PALMBLAD J., UDÉN A.-M. & VENIZELOS N. 1980. Structure and function of neutrophil leukocytes from patients with the immotile cilia syndrome. *Acta Med Scand* 208, 145-154.
- BORGEAT P. & SAMUELSSON B. 1979. Arachidonic acid metabolism in polymorphonuclear leukocytes: Effects of lipophore A-3187. *Proc Natl Acad Sci USA* 76, 2148-2152.
- CHENOWETH D. E., ROWE, J. G. & HYLI T. E. 1979. A modified method for chemotaxis under agarose. *J Immunol Methods* 25, 337-353.
- FORD-HUTCHINSON A. W., BRAY M. A., DOIG M. W., SHIPLEY M. E. & SMITH M. J. H. 1980. Leukotriene B₄ - potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* 286, 264-265.
- GOETZL, E. J. 1980. The unique roles of mono-hydroxy-o-octetetranoic acids (HETEs) in the regulation of human eosinophil function. / The eosinophil. Chemical, biochemical and functional aspects. Proceedings of the Eosinophil Centennial (ed. A. Mahmoud & K. F. Austen), Grune and Stratton, New York, in press.
- HANSSON O. & RÅDMARK O. 1980. Leukotriene C₄. Isolation from human polymorphonuclear leukocytes. *FEBS Lett* (submitted for publication).
- HEDQVIST P., DAHLÉN S. E., GUSTAVSSON L., HAMMARSTRÖM S. & SAMUELSSON B. 1980. Biological profile of leukotrienes C₄ and D₄. *Acta Physiol Scand* 110, 331-333.
- RÅDMARK O., MALMSTEN C. & SAMUELSSON B. 1980. Leukotriene A₄. Stereochemistry and enzymatic conversion to leukotriene B₄. *Biochem Biophys Res Commun* 92: 954-961.
- SAMUELSSON B. & HAMMARSTRÖM, S. 1980. Nomenclature for leukotrienes. *Prostaglandins* 19, 645-648.
- SAMUELSSON B., HAMMARSTRÖM, S., MURPHY R. C. & BORGEAT P. 1980. Leukotrienes and slow reacting substance of anaphylaxis. *Allergy* 35, 375-381.
- SAMUELSSON B., FOLCO O., GRANSTRÖM E., KRYDHAL, W. & MALMSTEN C. 1978. Prostaglandins and thromboxanes. *Ann Rev Biochem* 47, 997-1029.

Table 1 The ability of LTB₄, LTC₄, 5-HETE and compounds I and II to induce a stimulated migration of neutrophils measured as the distance to the leading front cells in mm

The unstimulated migration never exceeded 0.13 mm in any examination. Mean \pm S.D. values for PMNLs from 5 different subjects (each sample triplets)

Conc. (M)	LTB	LTC	5-HETE	Compound I	Compound II	fMLP
10 ⁻⁶	0.61 \pm 0.09*	0.17 \pm 0.02	0.18 \pm 0.03	0.66 \pm 0.30*	0.74 \pm 0.28*	-
10 ⁻⁵	0.81 \pm 0.05	0.14 \pm 0.04	0.13 \pm 0.01	0.43 \pm 0.07*	0.71 \pm 0.14	-
10 ⁻⁴	0.56 \pm 0.07*	0.13 \pm 0.02	0.13 \pm 0.05	0.10 \pm 0.01	0.20 \pm 0.02	0.80 \pm 0.27*
10 ⁻³	0.27 \pm 0.01	0.17 \pm 0.02	0.10 \pm 0.03	0.13 \pm 0.04	0.13 \pm 0.04	-
10 ⁻²	0.13 \pm 0.02	0.11 \pm 0.02	0.14 \pm 0.04	0.11 \pm 0.03	0.11 \pm 0.03	-

* $P < 0.01$ $P < 0.05$ in comparison with measures for unstimulated cells (Wilcoxon's signed rank test)

PMNLs showed a desensitized pattern of stimulated migration (Chenoweth et al. 1979)

Mean of triplets

containing approximately 10×10^6 cells/l was filled in the central well. The chemotactic factor either fMLP (10^{-7} M) in Hank's medium, 5-HETE or the leukotrienes dissolved in 10% ethanol were filled in the outer well. Tissue culture medium was added to the inner well.

The dishes were incubated for 0.5–3 h at 37°C in an atmosphere of 5% CO₂. After fixation with methanol and staining with hematoxylin and fuchsin the agarose dishes were removed and the distance migrated by the front was measured by microscopy and given in mm.

The orientation of lamellopodia and nuclei of 300 PMNLs migrating towards the cytotoxin and tissue medium wells was estimated by microscopy. The cells were considered polarized in the gradient when the nuclei were in the rear and the anterior lamellopodia located within a 90° sector open towards the cytotoxin well (Afzelius et al. 1980).

Effects of incubation of PMNLs with indomethacin or eicosatetraynoic acid (ETYA). When migration of PMNLs was stimulated with 10^{-7} M fMLP preincubation for 15 min with indomethacin (2.5×10^{-5} – 2.5×10^{-6} M) caused dose dependent inhibition of migration. Spontaneous migration was also slightly impaired ($P < 0.05$). However when the concentration of indomethacin was decreased to 2.5×10^{-8} M a significant enhancement of fMLP stimulated migration was observed ($P < 0.01$, Wilcoxon's signed rank test). In concentrations 2.5×10^{-7} M or below indomethacin showed no significant effect on stimulated or spontaneous migration when compared to controls.

PMNLs pretreated with ETYA for 15 min at 37°C showed markedly impaired migration when final inhibitor concentration was 2.5×10^{-4} – 2.5×10^{-5} M.

However at 2.5×10^{-4} M ETYA many PMNLs seemed damaged according to results of trypan blue exclusion. At final concentrations 2.5×10^{-4} M or below the results were comparable to those of control cells. This occurred both when migration was spontaneous or stimulated with 10^{-7} M fMLP.

Effects of 5 HETE, LTC₄, LTB₄ and compounds I and II. When the different compounds to be tested were added to the cytotoxin well PMNL migration was significantly stimulated by LTB₄ ($P < 0.01$, Wilcoxon's signed rank test) and compounds I and II ($P < 0.05$) (Table 1). When 5 HETE or LTC₄ were utilized the migration was not significantly different from that of control cells.

In order to assess whether LTB₄ and compounds I and II stimulated directed or random migration, i.e. chemotaxis or chemokinesis, we analyzed the course of migration and the degree of orientation at 30, 60 and 120 min after the start of incubation and compared this with fMLP in cytotoxin. PMNLs stimulated by fMLP showed both the highest degree of orientation and progression of the cell front at 30 and 60 min. The orientation decreased after 120 min to control values (reference range (± 2 S.D.) is 13.5–30.9%, Palmblad et al. to be published).

PMNLs stimulated by LTB₄ also showed directed and stimulated migration at 30 and 60 min when concentrations were 10^{-6} – 10^{-7} M. At 10^{-8} M LTB₄ decreased the stimulated migration presumably by causing desensitization (Chenoweth et al. 1979).

With compound I directed migration was observed at concentrations of 10^{-6} – 10^{-8} M and with compound II at concentrations of 10^{-6} – 10^{-7} M in the system employed.

The observation that eicosatetraynoic acid

Acta

Vol 110

Physiologica Scandinavica

Editorial Board

P. Kruheffer
Lundberg

M. R. Bangstrom
Helsinki

J. Jansen
Oslo

Y. Zotterman
Stockholm

U. S. von Euler
(Editor) Stockholm

Contents

Vol. 110, No. 1 September 1980

- 1 JOHANSSON B. B. & HENNING M. 6-hydroxydopamine and the blood-brain barrier in adult conscious rats
- 3 JÄRHULT I., ANDERSSON P.-O., HOLST I., MOGHIMZADEH E. & NOBIN A. On the sympathetic innervation to the cat's liver and its role for hepatic glucose release
- 11 DAHLSTRÖM A., BÖÖJ S., HETWALL P.-O. & LARSSON P. A. The effect of chronic nicotine and withdrawal on intrabronchial dynamics of acetylcholine and related enzymes in a preganglionic neuron system of the rat
- 1 EKSTRÖM J. & ELMÉR M. Compensatory increase of responses to nerve stimulation of the partially denervated rat urinary bladder
- 31 UDDMAN R., FAHRENKRUG J., MALM L., ALUMETS J., HÅKANSON R. & SUNDLER F. Neuronal VIP in salivary glands. Distribution and release
- 39 KEDINÄNEN M., KUUKKA J., WISELJUS S. J., SUOLINNA E. M., SOLIN O., LÄNGSTRÖM B. & NÄNTÖ V. Tissue capillary exchange and distribution of carbon-11 labelled ethanol in the isolated perfused rat liver
- 47 NEVALAINEN T. O., HAKUMÄKI M. O. K., HYÖDYNMÄÄ S. J., NÄRHI M. V. O. & SARAJÄS H. S. S. Distension of pulmonary vein—left atrial junction. Heart rate responses in conscious and anesthetized dogs
- 53 LARSSON M., JOHNSON L., NYLANDER, G. & ÖHMAN U. Plasma water and ⁵¹Cr-EDTA equilibration volumes of different tissues in the rat

- gland and nasal mucosa. Possible interaction with VIP
- 703 AUER, L. M. & JOHANSSON B. III Pial venous constriction during cervical sympathetic stimulation in the cat
- 707 EDIN R., LUNDBERG J. M., LIDBERG P., DAHLSTRÖM A. & AHLBÄCK H. Atropine sensitive contractile motor effects of substance P on the feline pylorus and stomach in vivo
- 711 SOLLEVI A. The sensitivity of perfused fat cells to the antipolytic action of adenosine
- 715 NILSSON H. & FOLKOW B. Structurally reduced compliance of the venous capacitance vessels in spontaneously hypertensive rats (SHR)
- 719 HANBERG M., HEDQVIST P. & RÅDE GRAN K. Identification of 15-hydroxy 5,8,11-13-cicosatetraenoic acid (15-HETE) as a major metabolite of arachidonic acid in human lung
- 723 CARLSON H. & SKOGLUND C. R. Esteroceptive influence from trunk region on mono-synaptic head limb reflexes in the cat
- Appendix Supplement 21*
- Supplementum 487* JANSSON E. Diet and muscle metabolism in man with reference to fat and carbohydrate utilization and its regulation
- Vol 110 No. 3 November 1980
- 725 SIENKOWICZ E. & GJEDDE A. Post ischemic coma in rat: effect of different pre-ischemic blood glucose levels on cerebral metabolic recovery after ischemia
- 733 HASIMARSTRÖM M. Endometrial cholinergic secretory responses during estrous cycle pregnancy and after estrogen and/or progesterone treatment of the guinea-pig
- 741 JOHANSSON M. & WOHLFART H. Cellular calcium as a determinant of action potential duration in rabbit myocardium
- 749 CLAUSEN G., HOPE A., KIRKEBØ A., TYSSEBØTN I. & AUKLAND K. Distribution of blood flow in the dog kidney. II. Saturation rates of inert diffusible tracers versus uptake of 15 μ m microspheres during vasodilation and vasoconstriction
- 759 FELLENIUS E., HEDBERG R. & KARLSSON M. The effect of β -receptor blockade on adrenaline-induced changes in redox and energy state in m. vastus lateralis and m. soleus of the rat in vivo
- 267 LINS P. E., PETERSSON B. & EFENDIĆ S. Effects of short term and prolonged infusions of somatostatin on endocrine pancreas, body weight and food intake in rats
- 277 HOHTOLA, E., SAARELA S. & HISSA R. Effects of blood pressure manipulations on shivering thermogenesis in the pigeon
- 285 HAUNSSÖ S. & AMTORP O. Regional blood flow during reactive hyperemia in canine myocardium as determined by local washout of Xenon-133
- 795 GJÖRSTRUP P. Taste and chewing as stimuli for the secretion of amylase from the parotid gland of the rabbit
- 303 KOIVUSALO F. Degradation of TRF immunoreactivity in rat hypothalamus, extra-hypothalamic brain and pancreas
- 307 HULTHÉN U. L., DYMLING J. F. & HÖKFELT B. Kinins in relation to kallikrein activity, kininogen, electrolytes, aldosterone and catecholamines in urine from normal individuals
- 315 AARS H. Central interaction between reflex responses to activity in aortic nerve A and C fibres
- Short Communications*
- 321 FUXE, K., GANTEN D., KÖHLER C., SCHULL, B. & SPECK, G. Evidence for differential localization of angiotensin-I converting enzyme and renin in the corpus striatum of rat
- 325 ROSELL, S. Experimental evidence for neurotension or a metabolite being a hormone
- 335 RÖKAEUS Å. & McDONALD T. Release of neurotensin-like immunoreactivity (NTLI) from rat intestine following administration of bombesin
- 327 THOR, K., RÖKAEUS Å., KAGER, L. & ROSELL, S. (Oln)-Neurotensin and gastrointestinal motility in man
- 329 KIHLE, B., RÖKAEUS Å., ROSELL, S. & OLBE, L. Inhibition of pentagastrin stimulated gastric acid secretion and rise in the plasma concentration of neurotensin-like immunoreactivity (NTLI) by intraduodenal oleic acid in man
- 330 WIKLUND B., RÖKAEUS Å., HALLBERG D. & ROSELL, S. Plasma NTLI after

- 59 HELLSTRAND P & ARNER A Contraction of the rat portal vein in hypertonic and isotonic medium mechanical properties and effects of Mg^{2+}
- 69 ARNER A & HELLSTRAND P Contraction of the rat portal vein in hypertonic and isotonic medium rates of metabolism
- 77 RICKSTEN S E & THORÉN P Reflex inhibition of sympathetic activity during volume load in awake normotensive and spontaneously hypertensive rats
- 83 METSÄKETELÄ T KUOSA R & VAAPATAALO H Temporal dissociation between the negative inotropism and the increase in cyclic GMP level induced by choline esters in spontaneously beating rat atria preparations
- 89 HELLSTRAND P & JÄRHULT J Effects of nine different gastrointestinal polypeptides on vascular smooth muscle in vitro
- 95 HALLBACK D A JODAL M & LUNDGREN O Villous tissue osmolality water and electrolyte transport in the cat small intestine at varying luminal osmolalities

Short Communications

- 101 SJÖBERG H E ARVER S & BUCHT E High concentration of immunoreactive calcitonin of prostatic origin in human semen
- 103 GRILLNER S & WALLÉN P Does the central pattern generation for locomotion in lamprey depend on glycine inhibition?
- 107 LUNDBERG J M HÖKFELT T ANGÅRD A KIMMEL J GOLDSTEIN M & MARKEY K Coexistence of an avian pancreatic polypeptide (APP) immunoreactive substance and catecholamines in some peripheral and central neurons
- 111 PELLIGRINO D & SIESJÖ B K The influence of circulating adrenaline on extracellular fluid potassium concentration in the brain

Appended Supplements

- Supplementum 485 EDIN R The vagal control of the pyloric motor function A physiological and immunohistochemical study in cat and man
- Supplementum 486. KVIST U Sperm nuclear chromatin decondensation ability An in vitro study on ejaculated human spermatozoa

Vol 110 No 2, October 1980

- 113 ÅLUND M Juxtaglomerular cell activity during hemorrhage and ischemia as revealed by quinacrine histofluorescence
- 123 LERNER U The effect of dibutyl cAMP and PGE_2 on lysosomal enzyme release and lactate production in relation to bone resorption in vitro
- 131 MARNIEMI J PELTONEN P VUORI I & HIETANEN E Lipoprotein lipase of human postheparin plasma and adipose tissue in relation to physical training
- 137 DELBRO D & LISANDER H Non-ganglionic cholinergic excitatory pathways in the sympathetic supply to the feline stomach An efferent system or afferents with excitatory axon collaterals?
- 145 BRISMAR T & JEFFERYS J G R Convulsants (benzylpenicillin and pentylenetetrazol) on potential clamped myelinated axons from the rat
- 149 ABDUL RAHMAN A & SIESJÖ B K Local cerebral glucose consumption during insulin-induced hypoglycemia and in the recovery period following glucose administration
- 161 EKELUND K & JOHANSSON C Output of bile and pancreatic enzymes after test meals with different fat content Influence of body weight on pancreatic enzyme composition
- 167 HAUGE A THORESEN M & WALLOE L Changes in cerebral blood flow during hyperventilation and CO_2 -breathing measured transcutaneously in humans by a bidirectional pulsed ultrasound doppler blood velocimeter
- 175 HISSA R GEORGE J C STEVENS E D & SAARELA S Thermoregulatory responses to tyramine in the pigeon
- 181 SELLIN L C LIBELIUS R LUNDQUIST I TÄGERUD H & THESLEFF S Membrane and biochemical alterations after denervation and during reinnervation of mouse skeletal muscle
- 187 HELLSTRAND E Morphological and histochemical properties of tongue muscles in cat

Short Communications

- 199 LUNDBERG J M ANGÅRD A HÖKFELT T & KIMMEL J Avian pancreatic polypeptide (APP) inhibits atropine resistant vasodilation in cat submandibular salivary gland

INDEX AUCTORUM

- 315 AARS, H. Baroreceptor A and C fibre inter-
action
- 149 ABDUL RAHMAN A & SIESJÖ B K
Cerebral glucose consumption
- 30 AHLMAN H see EDIN R.
- 31 ALUMETS J see UDDMAN R.
- 113 ÅLUND, M. Juxtaglomerular cell activity
- 337 ANTORP O Brain uptake kinetics
- 283 ANTORP O see HAUNSSÖ S
S ANDERSSON P-O see JÄRHULT J
- 107 ÅNGGÅRD A. see LUNDBERG J M
- 89 ÅNGGÅRD A. see LUNDBERG J M
- 19 ARNER, A. see HELLSTRAND P
- 19 ARNER, A. & HELLSTRAND P Portal vein
metabolism in hypertonicity
- 104 ARVER, S. see SJOBERG H E.
- 263 AUER, L. M. & JOHANSSON B B Sym-
pathetic stimulation and pial veins
- 248 AUKLAND K see CLAUSEN G
- 351 BECH C & JOHANSEN K. Blood flow in
deck thermal penning
- 13 BÖÖJ S. see DAHLSTRÖM A.
- 69 BRISMAR, T. Potential clamp of demyelinated
nerve
- 145 BRISMAR, T. & JEFFERYS J G R Pz
and PTZ on nodal membrane
- 101 BUCHT E. see SJOBERG H E.
- 223 CARLSON H & SKOGLUND C R Mono-
synaptic hind limb reflexes
- 113 CHAPMAN A. INGVAR, M. & SIE-
SÖ B K. Free fatty acids in status brain
- 49 CLAUSEN G HOPE, A. KIRKEBØ A.
TYSSEBØTN I & AUKLAND K Local
blood flow in the dog kidney
- 391 CRANWELL, P M see UVNAS-WALLEN-
STEN K.
- 31 DAHLÉN S E see HEDQVIST P
- 307 DAHLSTRÖM A. see EDIN R.
- 13 DAHLSTRÖM A BÖÖJ S HEIWAALL
P-O & LARSSON P A Nicotine on intra-
neural acetylcholine
- 177 DELBRO D & LISANDER, B Sympathetic
baric excitation
- 107 DYMLING J F see HULTHÉN U L.
- 30 EDIN R. LUNDBERG J M LIDBERG P
- DAHLSTRÖM A & AHLMAN H Motility
stomach pylorus, substance P and cat
- 375 EDVINSSON L. see HARDEBO J E.
- 267 EFENDIĆ S see LINS P E.
- 391 EFENDIĆ, S see UVNAS-WALLEN-
STEN K.
- 161 EKELUND K & JOHANSSON C Bile and
pancreatic enzymes after test meals
- 1 EKSTRÖM J & ELMÉR, M Collateral
sprouting in urinary bladder
- 21 ELMÉR, M see EKSTRÖM J
- 449 ENGSTEDT L. see MALMISTEN C. L.
- 31 FAHRENKRUG J see UDDMAN R.
- 343 FASTH S. HULTÉN L. & NORDGREN B
Hepatic and intestinal blood flow
- 259 FELLENIUS E HEDBERG R. & KARLS-
SON N Beta receptor blockade on muscle
energy state
- 419 FLEMSTRÖM G & GARNER, A Ca and
gastric secretions
- 215 FOLKOW B see NILSSON H
- 321 FUXE K GANTEN D KOHLER, C
SCHÜLL, B & SPECK G Angiotensin-1
converting enzyme and renin in brain
- 357 GABELLA G see UVELIUS B
- 371 GANTEN D see FUXE, K
- 419 GARNER, A. see FLEMSTRÖM G
- 175 GEORGE, J C see HISSA R.
- 225 GJEDDE, A see SIEMKOWICZ, E.
- 295 GJØRSTRUP P Reflex secretion of amylase
- 107 GOLDSTEN M see LUNDBERG J M.
- 103 GRILLNER, S. & WALLÉN P Glycine and
locomotion in lamprey
- 331 GUSTAFSSON L. see HEDQVIST P
- 401 GUSTAFSSON L. HEDQVIST P &
LUNDGREN G Prostaglandin on ACh re-
lease
- 31 HÅKANSON R. see UDDMAN R
- 47 HAKUMÄKI M O K. see NEVALAI-
NEN T O
- 330 HALLBERG D see WIKLUND B
- 95 HALLBÄCK D-A JODAL M & LUND-
GREN O Lumen and tissue osmolality in gut
- 219 HAMBERG M. HEDQVIST P & RÅDE-
GRAN K Arachidonic acid transformation
in human lung

administration of fat to obese volunteers and patients operated with jejuno-ileal bypass

- 331 HEDQVIST P DAHLÉN S E GUSTAFSSON L HAMMARSTRÖM S & SAMUELSSON B Biological profile of leukotrienes C and D₄
- 335 CHAPMAN A INGVAR M & SIE SJÖ B K Free fatty acids in the brain in bicuculline induced status epilepticus

Appended Supplement

Supplementum 488 RIDDERSTRÅLE Y Intracellular localization of carbonic anhydrase in some vertebrate nephrons

Vol 110 No 4 December 1980

- 337 AMTORP O Estimation of capillary permeability of mulin sucrose and mannitol in rat brain cortex
- 343 FASTH S HULTÉN L & NORDGREN S Adjustments of hepatic and small intestine blood flow on selective vasoconstrictor fibre stimulation
- 351 BECH C & JOHANSEN K Blood flow changes in the duck during thermal panting
- 357 UVELIUS B & GABELLA G Relation between cell length and force production in urinary bladder smooth muscle
- 367 LARSEN J A & THOMSEN O Ø Vana date induced oliguria and vasoconstriction in the cat
- 375 HARDEBO J E EDVINSSON L & JOHANSSON B B Cerebrovascular sympathetic denervation and blood brain barrier function in conscious rats
- 381 SJÖQVIST A & LJUNG B Dissociation of electrical and mechanical activity caused by vibrations in the spontaneously active smooth muscle of the rat portal vein
- 385 LEHTO H & TIRRI R Characterization of contractions in mechanically disaggregated myocardial cells from the rat
- 391 UYNÄS-WALLÉN K EFENDIĆ B JOHANSSON C SJÖDIN L & CRANWELL P D Effect of intraluminal pH on the

release of somatostatin and gastrin into atrial, bulbar and ileal pouches of conscious dogs

- 401 GUSTAFSSON L HEDQVIST P & LUNDGREN G Pre and postjunctional effects of prostaglandin E₂ prostaglandin synthetase inhibitors and atropine on cholinergic neurotransmission in guinea pig ileum and bovine iris
- 413 RICKSTEN S E YAO T LJUNG B & THORÉN P Distensibility of left atrium in normotensive and spontaneously hypertensive rats
- 419 FLEMSTRÖM G & GARNER A Stimulation of gastric acid and bicarbonate secretions by calcium in guinea pig stomach and amphibian isolated mucosa

Short Communications

- 427 WAHLSTRÖM A & TERENIUS L Factor in human CSF with apparent morphine antagonistic properties
- 431 THORÉN P Characteristics of right ventricular receptors with non-medullated vagal afferents in the cat
- 435 REHNCRONA S ROSEN I & SIESJÖ B K Excessive cellular acidosis an important mechanism of neuronal damage in the brain
- 439 BRISMAR T Potential clamp of demyelinated rat nerve fibres
- 441 HEDQVIST P Autoinhibition versus trans synaptic control of noradrenaline secretion in the rabbit kidney
- 445 THOREBJÖRK H E & OCHOA J L Specific sensations evoked by activity in single identified sensory units in man
- 449 MALMSTEN C L PALMBLAD J UDÉN A M RÅDMARK O ENGSTEDT L & SAMUELSSON B Leukotriene B A highly potent and stereospecific factor stimulating migration of polymorphonuclear leukocytes

Appended Supplement

Supplementum 489 GUSTAFSSON L E Studies on modulation of transmitter release and effector responsiveness in autonomic cholinergic neurotransmission

INDEX AUCTORUM

- 315 AARS, H. Baroreceptor A- and C fibre inter-
action
- 149 ABDUL-RAHMAN A. & SIESJÖ B. K.
Cerebral glucose consumption
- 257 AHLMAN H. see EDIN R.
- 31 ALUMETS, J. see UDDMAN R.
- 113 ÅLUND M. Juxtaglomerular cell activity
- 337 ÅMSTORP O. Brain uptake kinetics
- 285 ÅMSTORP O. see HAUNSSÖ B.
J. ANDERSSON P.-O. see JÄRHULT J.
- 107 ÅNGGÅRD A. see LUNDBERG J. M.
- 109 ÅNGGÅRD A. see LUNDBERG J. M.
- 19 ÅRNER, A. see HELLSTRAND P.
- 69 ÅRNER, A. & HELLSTRAND P. Portal vein
metabolism in hypertension
- 101 ÅRVER, S. see SJÖBERG H. E.
- 213 AUER, L. M. & JOHANSSON B. B. Sym-
pathetic stimulation and portal veins
- 49 AUKLAND K. see CLAUSEN G.
- 31 BECH, C. & JOHANSEN K. Blood flow in
duck thermal panting
- 13 BÖÖJ S. see DAHLSTRÖM A.
- 49 BRISVIAR, T. Potential clamp of demyelinated
nerve
- 105 BRISVIAR, T. & JEFFERYS J. O. R. Pz
and PTZ on nodal membrane
- 101 BUCHT E. see SJÖBERG H. E.
- 223 CARLSON H. & SKOGLUND C. R. Mono-
synaptic hind limb reflexes
- 115 CHAPMAN A. INGVAR, M. & SIE-
SÖ B. K. Free fatty acids in states brain
- 49 CLAUSEN G. HOPE, A. KIRKEBØ A.
TYSSEBØT N. & AUKLAND K. Local
blood flow in the dog kidney
- 101 CRANWELL, P. D. see UVNÄS-WALLÉN-
STEN K.
- 31 DAHLEN S. E. see HEDQVIST P.
- 27 DAHLSTRÖM A. see EDIN R.
- 13 DAHLSTRÖM A. BÖÖJ S. HEIWA-
LL, P.-O. & LARSSON P. A. Nicotine on intra-
neuronal acetylcholine
- 117 DELBRO D. & LISANDER B. Sympathetic
gastroc excitation
- 107 DYMLING J. F. see HULTHÉN U. L.
- 27 EDIN R. LUNDBERG J. M. LIDBERG P.
- DAHLSTRÖM A. & AHLMAN H. Motility
stomach, pylorus substance P and cat
- 375 EDVINSSON L. see HARDEBO J. E.
- 267 EFENDIĆ, S. see LINS P. E.
- 391 EFENDIĆ, S. see UVNÄS-WALLÉN-
STEN K.
- 161 EKELOUND K. & JOHANSSON C. Bile and
pancreatic enzymes after test meals
- 21 EKSTRÖM J. & ELMÉR M. Collateral
sprouting in urinary bladder
- 21 ELMÉR M., see EKSTRÖM J.
- 449 ENGSTEDT L. see MALMSTEN C. L.
- 31 FAHRENKRUG J. see UDDMAN R.
- 343 FASTH S. HULTÉN L. & NORDGREN S.
Hepatic and intestinal blood flow
- 459 FELLENIUS E. HEDBERG R. & KARLS-
SON N. Beta receptor blockade on muscle
energy state
- 419 FLEMSTRÖM G. & GARNER, A. Ca²⁺ and
gastric secretions
- 215 FOLKOW B. see NILSSON H.
- 321 FUXE, K. GANTEN D. KÖHLER C.
SCHULL, B. & SPECK G. Angiotensin-
converting enzyme and renin in brain
- 357 GABELLA G. see UVELIUS B.
- 31 GANTEN D. see FUXE, K.
- 419 GARNER, A. see FLEMSTRÖM G.
- 175 GEORGE, J. C. see HISSA R.
- 225 GJEDDE A. see STEMKOWICZ, E.
- 295 GJÖRSTRUP P. Reflex secretion of amylase
- 107 GOLDSTEN M. see LUNDBERG J. M.
- 103 GRILLNER, S. & WALLÉN P. Glycine and
locomotion in lamprey
- 331 GUSTAFSSON L. see HEDQVIST P.
- 401 GUSTAFSSON L. HEDQVIST P. &
LUNDGREN G. Prostaglandin on ACh re-
lease
- 31 HÄKANSON R. see UDDMAN R.
- 47 HAKUMÄKI M. O. see NEVALAINEN T. O.
- 330 HALLBERG D. see WIKLUND B.
- 95 HALLBÄCK D. A. JODAL, M. & LUND-
GREN O. Lumen and tissue osmolality in gut
- 119 HAMBERG M. HEDQVIST P. & RÄDE-
GRAN K. Arachidonic acid transformation
in human lung

- 233 HAMMARSTRÖM M Secretory responses in reproductive cycle
- 331 HAMMARSTRÖM S see HEDQVIST P
- 375 HARDIBO J F EDVINSSON L & JOHANSSON B B Blood brain barrier in conscious rats
- 167 HAUGE A THORESEN M & WALLOE L Brain blood flow measured by ultrasound
- 785 HAUNSO S & AMTORP O Reactive hyperemia in canine myocardium
- 259 HEDBERG R see FELIENIUS I
- 441 HEDQVIST P Feedback control of NA secretion
- 401 HEDQVIST P see GUSTAFSSON L
- 719 HEDQVIST P see HAMBERG M
- 331 HEDQVIST P DAHLÉN S F GUSTAFSSON L HAMMARSTRÖM S & SAMUELSSON B Biological actions of leukotrienes C₄ and D
- 13 HEIWALL P-O see DAHLSTRÖM A
- 187 HELLSTRAND F Properties of cat tongue muscles
- 59 HELLSTRAND P & ARNER A Portal vein mechanics in hypertension
- 69 HELLSTRAND P see ARNER A
- 89 HELLSTRAND P & JÄRHULT J Peptides and vascular smooth muscle
- 1 HENNING M see JOHANSSON B B
- 39 HESELIUS S J see KEINANEN M
- 131 HIETANEN E see MARNIEMI J
- 777 HISSA R see HOHTOLA E
- 175 HISSA R GEORGE J C STEVENS F D & SAARELA S Thermoregulatory responses to tyramine in the pigeon
- 77 HOHTOLA E SAARELA S & HISSA R Shivering thermogenesis in pigeon
- 307 HÖKFELT B see HULTHÉN U L
- 107 HÖKFELT T see LUNDBERG J M
- 199 HÖKFELT T see LUNDBERG J M
- 5 HOLST J see JÄRHULT J
- 749 HOPE A see CLAUSEN G
- 743 HULTÉN L see FASTH S
- 307 HULTHÉN U L DYMLING J F & HÖKFELT B Kinins in urine
- 47 HYÖDYNMAA S J see NEVALAINEN T O
- 335 INGVAR M see CHAPMAN A
- 89 JÄRHULT J see HELLSTRAND P
- 5 JÄRHULT J ANDERSSON J HOLST J MOGHIMZADEH E. & BIN A Sympathetic nerves to the feline
- 145 JIFFERYS J G R see BRISMAR, T
- 95 JODAL M see HALLBÄCK D A
- 351 JOHANSEN K see BECH C
- 703 JOHANSSON B B see AUER L M
- 161 JOHANSSON C see EKELOUND A
- 375 JOHANSSON B B see HARDEBO J
- 1 JOHANSSON B B & HENNING J hydroxydopamine and blood-brain barrier
- 391 JOHANSSON C see UVNÄS-WALLSTEN K
- 741 JOHANSSON M & WOHLFART Action potential duration in rabbit heart
- 53 JOHNSON L see LARSSON M
- 377 KAGFR L see THOR K
- 259 KARLSSON N see FELIENIUS E
- 39 KEINANEN M KUIKKA J HILJUS S J SUOLINNA E M SOJAN LÄNGSTRÖM B & NÄNTÖ V Ethanol change in the liver
- 379 KIHU B RÖKAEUS Å ROSFELT OLBE L Oleic acid on plasma neurotensin
- 107 KIMMEL J see LUNDBERG J M
- 199 KIMMEI J see LUNDBERG J M
- 749 KIRKEBO A see CLAUSEN G
- 371 KÖHLER C see GUXE K
- 303 KOIVUSALO F Degradation of immunoreactivity
- 39 KUIKKA J see KEINANEN M
- 83 KUOSA H see METSA-KITTELA T
- 39 LÄNGSTRÖM B see KEINANEN M
- 367 LARSEN J A & THOMSEN O O V date kidney function and hemodynamics
- 53 LARSSON M JOHNSON L NYLÉN G & ÖHMAN U EDTA Space different tissues
- 13 LARSSON P A see DAHLSTRÖM A
- 123 LERNER U Cyclic AMP and mechanisms bone resorption
- 385 LETHO H & TIRRI R Contraction disaggregated myocardial cells
- 181 LIEBOWITZ R see SPILIN L C
- 707 LIDBERG P see LIDIN H
- 767 LINS P F PETERSSON B & PITTMAN S Somatostatin on endocrine pancreas
- 137 LISANDER B see DELBRO H
- 413 LJUNG H see RICKSTEN S L.

- LUNDG, B. see SJÖQVIST A.
 LUNDBERG, J. M. ÅNGGÅRD A. HOK
 FELT T. & KIMMEL, J. Avian pancreatic
 polypeptide inhibiting vasodilatation
 LUNDBERG, J. M. see EDIN R.
 LUNDBERG, J. M. HOKFELT T. ÅNG-
 GÅRD, A. KIMMEL, J. GOLDSTEIN M.
 & MARKEY K. Pancreatic polypeptide and
 catecholamines in some neurons
 LUNDGREN G. see GUSTAFSSON L.
 LUNDGREN O. see HALLBÄCK D. A.
 LUNDQVIST L. see SELLIN L. C.
 McDONALD T. see ROKAEUS Å.
 VALM, L. see UDDMAN R.
 VALNSTEN C. L., PALMBLAD J.
 EDEN A.-M. RÅDMARK O. ENG-
 STEDT L. & SAMUELSSON B. Leuko-
 cyte B4 and leukocyte migration
 MARKEY K. see LUNDBERG J. M.
 MARNIEMI J. PELTONEN P. VUORI J.
 & HIETANEN E. Lipoprotein lipase and
 physical training
 METSÄ-KETELA T. KUOSA R. &
 PAPAATALO H. Atrial isothermism and cyclic
 GMP
 MOGHIMZADEH E. see JÄRHULT J.
 NÄNTÖ V. see KEINANEN M.
 NARHI M. V. O. see NEVALAINEN T. O.
 NEVALAINEN T. O. HAKUMAKI M.
 O. K. HYÖDYNNÄÄ, S. J. NARHI M. V.
 O. & SARAJAS H. S. S. Pulmonary vein dis-
 tension and heart rate
 NILSSON H. & FOLKOW B. Reduced
 ocular compliance in SHR
 NOBIN A. see JÄRHULT J.
 NORDGREN B. see FÄSTH S.
 NYLANDER, G. see LARSSON M.
 OCHOA J. L. see TOREBJÖRK H. E.
 ÖHMANN U. see LARSSON M.
 OLBE, L. see KIHIL, B.
 PALMBLAD J. see MALMISTEN C. L.
 PELLIGRINO D. & SIESJÖ B. K. Adrena-
 line and brain (K.)
 PELTONEN P. see MARNIEMI J.
 PETERSSON B. see LINS P. E.
 RÅDEGRAN K. see HANBERG M.
 RÅDMARK O. see MALMISTEN C. L.
 435 REHNCRONA S. ROSÉN I. & SIE
 SJÖ B. K. Brain damage by acidosis
 77 RICKSTEN S. E. & THORÉN P. Sym-
 pathetic activity in hypertensive rats
 413 RICKSTEN S. E. YAO T. LJUNG B. &
 THORÉN P. Distensibility of left atrium in
 rats
 329 RÖKAEUS Å. see KIHIL, B.
 376 ROKAEUS Å. & McDONALD T. Neuro-
 tensin release after bombesin
 377 ROKAEUS Å. see THOR, K.
 330 ROKAEUS Å. see WIKLUND B.
 325 ROSELL, S. Neurotensin as hormone
 379 ROSELL, S. see KIHIL, B.
 377 ROSELL, S. see THOR, K.
 435 ROSÉN I. see REHNCRONA S.
 330 ROSELL, S. see WIKLUND B.
 175 SAARELA S. see HISSA R.
 277 SAARELA S. see HOHTOLA E.
 331 SAMUELSSON B. see HEDQVIST P.
 449 SAMUELSSON B. see MALMISTEN C. L.
 47 SARAJAS H. S. S. see NEVALAINEN
 T. O.
 3 I. SCHÖLL, B. see FUXE K.
 181 SELLIN L. C. LIBELIUS R. LUND-
 QUIST I. TÄGERUD S. & THESLEFF S.
 Muscle alterations by denervation and reinner-
 vation
 225 SIEMKOWICZ, E. & GJEDDE, A. Post
 ischemic coma
 149 SIESJÖ B. K. see ABDUL RAHMAN A.
 335 SIESJÖ B. K. see CHAPMAN A.
 111 SIESJÖ B. K. see PELLIGRINO D.
 435 SIESJÖ B. K. see REHNCRONA, S.
 101 SJÖBERG H. E. ARVER, S. & BUCHT E.
 Calcitonin in human semen
 391 SJÖDIN L. see UYNAS-WALLEN-
 STEN K.
 381 SJÖQVIST A. & LJUNG B. Electromechani-
 cal dissociation by brations
 223 SKOGLUND C. R. see CARLSSON H.
 39 SOLIN O. see KEINANEN M.
 11 SOLLEVI A. Antipolytic potency of
 adenosine
 371 SPFCK, G., see FUXE K.
 175 STEVENS, E. D. see HISSA R.
 31 SUNDLER, F. see UDDMAN R.
 39 SUOLINNA, E. M. see KEINANEN M.
 181 TÄGERUD S. see SELLIN L. C.

- 233 HAMMARSTRÖM M Secretory responses in reproductive cycle
- 331 HAMMARSTRÖM S see HEDQVIST P
- 375 HARDEBO J E EDVINSSON L & JOHANSSON B II Blood brain barrier in conscious rats
- 167 HAUGE A THORESEN M & WAL LÖE L Brain blood flow measured by ultrasound
- 785 HAUNSO B & AMTORP O Reactive hyperemia in canine myocardium
- 259 HEDBERG R see FELLENIUS E
- 441 HEDQVIST P Feedback control of NA secretion
- 401 HEDQVIST P see GUSTAFSSON L
- 219 HEDQVIST P see HAMBERG M
- 331 HEDQVIST P DAHLÉN S E GUSTAFSSON L HAMMARSTRÖM S & SAMUELSSON B Biological actions of leukotrienes C and D₅
- 13 HEIWall P O see DAHLSTRÖM A
- 187 HELLSTRAND C Properties of cat tongue muscles
- 59 HELLSTRAND P & ARNER A Portal vein mechanics in hypertonicity
- 69 HELLSTRAND P see ARNER A
- 89 HELLSTRAND P & JÄRHULT J Peptides and vascular smooth muscle
- 1 HENNING M see JOHANSSON B II
- 39 HESELIUS S J see KEINANEN M
- 131 HIETANEN E see MARNIEMI J
- 777 HISSA R see HOHTOLA E
- 175 HISSA R GEORGE J C STEVENS E D & SAARELA B Thermoregulatory responses to tyramine in the pigeon
- 277 HOHTOLA E SAARELA S & HISSA R Shivering thermogenesis in pigeon
- 307 HÖKFELT B see HULTHÉN U L
- 107 HÖKFELT T see LUNDBERG J M
- 199 HÖKFELT T see LUNDBERG J M
- 5 HOLST J see JÄRHULT J
- 249 HOPE A see CLAUSEN G
- 343 HULTÉN L see FASTH S
- 307 HULTHÉN U L DYMLING J F & HÖKFELT B Kinins in urine
- 47 HYÖDYNMAA S J see NEVALAINEN T O
- 335 INGVAR M see CHAPMAN A
- 89 JÄRHULT J see HELLSTRAND P
- 5 JÄRHULT J ANDERSSON P HOLST J MOGHIMZADEH E & BIN A Sympathetic nerves to the feline
- 145 JEFFERYS J G R see BRISMAR, T
- 95 JODAL M see HALLBÄCK D A
- 351 JOHANSEN K see BECH C
- 703 JOHANSSON B II see AUER L M
- 161 JOHANSSON C see EKElund K
- 175 JOHANSSON B B see HARDEBO J E
- 1 JOHANSSON B B & HENNING M hydroxydopamine and blood-brain barrier
- 391 JOHANSSON C see UVNÄS-WALLSTEN K
- 741 JÖHANNSSON M & WOHLFART Action potential duration in rabbit heart
- 53 JOHNSON L see LARSSON M
- 377 KÄGER L see THOR K
- 259 KARLSSON N see FELLENIUS E
- 39 KEINANEN M KUIKKA J HEI LIUS S J SUOLINNA E M SOLIN LÄNGSTRÖM B & NANTÖ V Ethanol change in the liver
- 379 KIHl B RÖKAFUS Å ROSELL S OLBE L Oleic acid on plasma neurotensin
- 107 KIMMEL J see LUNDBERG J M
- 199 KIMMEL J see LUNDBERG J M
- 749 KIRKCHO A see CLAUSEN G
- 371 KÖHLER C see FUXE K
- 303 KOIVUSALO F Degradation of T_H immunoreactivity
- 39 KUIKKA J see KEINANEN M
- 83 KUOSA R see METSA KETELÄ T
- 39 LÄNGSTRÖM B see KEINANEN M
- 167 LARSEN J A & THOMSEN O O Væ date kidney function and hemodynamics
- 53 LARSSON M JOHNSON L NYLÄ DER G & ÖHMÄN U EDTA Space different tissues
- 13 LARSSON P A see DAHLSTRÖM A
- 123 LERNER U Cyclic AMP and mechanism bone resorption
- 385 LETHO H & TIRRI M Contractions disaggregated myocardial cells
- 181 LIBELIUS R see SELLIN L C
- 707 LIDBERG P see EDIN R
- 767 LINS P E PETERSSON B & EFENDI S Somatostatin on endocrine pancreas
- 137 LISANDER B see DELBRO D
- 413 LJUNG B see RICHSTEN S E

- 427 TERENIUS L *see* WAHLSTRÖM A
 181 THESLEFF S *see* SELLIN L C
 367 THOMSEN O Ø *see* LARSEN J A
 327 THOR K RÖKAEUS Å KÄGER L &
 ROSELL S Neurotensin and gastrointestinal
 motility
 445 TOREBJÖRK H E & OCHOA J L. Sen-
 sations mediated by single sensory units
 431 THORÉN P Right ventricular receptors
 77 THORÉN P *see* RICKSTEN S E
 413 THORÉN P *see* RICKSTEN S E
 167 THORESEN M *see* HAUGE A
 385 TIRRI R *see* LETHO H
 249 TYSSEBOTN I *see* CLAUSEN G
 449 UDÉN A M *see* MALMSTEN C L
 31 UDDMAN R FAHRENKRUG J
 MALM L ALUMETS J HÅKANSON R
 & SUNDLER F VIP in salivary glands
 357 UVELIUS B & GABELLA G
 bladder muscle
 391 UVNÄS-WALLENSTEN K EFENDI
 JOHANSSON C SJÖDIN L &
 WELL, P D Somatostatin and gastrin
 into dogs
 427 WAHLSTRÖM A & TERENIUS, L
 phine antagonistic factor in CSF
 103 WALLÉN P *see* GRILLNER S
 167 WALLOE L *see* HAUGE A
 83 VAPAATALO H *see* METSÄ KETE
 330 WIKLUND B RÖKAEUS Å H
 BERG D & ROSELL S Plasma
 tensin in obesity
 241 WOHLFART B *see* JÖHANSSON
 131 VUORI I *see* MARNIEMI J
 413 YAO T *see* RICKSTEN S E

